ELECTRIC FISH INVESTIGATION

Final Report

Contract

8 March 1973

258

ILLUSTRATIONS

Figure		Page
1	Mauthner cells	4
2	Electroreceptors	6
3	Cranial nerves of Electrophorus	8
4	Microelectrode amplifier	13
5	Microelectrode amplifier: schematic diagram	13
6	Loss in -dB with increasing frequency	14
7	Loss in -dB with increasing repetition rate	15
8	Device for sharpening metal electrodes	17
9	Device for applying silver chloride	17
10	Potentiometric device	19
11	Device for measuring microelectrode resistance	19
11A	Amplification factor, square wave	20
11B	Amplification factor, sinusoidal wave	21
11C	Recordings at different square wave	22
11D	Recordings at different square wave	23
12	Adjustable Lucite Tray	27
13	Instrumentation for Anaesthesia	27
14	Solution 1:10000 in water on Sternarchus albifrons	29
15	Sternarchus albifrons #7 normal electrical activity	31
16	Sternarchus albifrons #7 in anesthetic tricaine methane sulfonate, 1 minute	31
17	Sternarchus albifrons #7 in anesthetic tricaine methane sulfonate, 2 minutes	31
18	Sternarchus albifrons #7 in anesthetic tricaine methane sulfonate, 4 minutes	31
19.	Sternarchus albifrons #7 in anesthetic tricaine methane sulfonate, 5 minutes	32
20.	Sternarchus albifrons recuperating, 5 minutes	32

...

Figure		Page
21	Sternarchus albifrons recuperating, 8 minutes	32
22	Set-up for microelectrode	32
23	Sternarchus albifrons No. 1 just before anaesthesia	33
24	Sternarchus albifrons No. 1 anaesthetized with thiopental sodium (2 min.)	33
25	Sternarchus albifrons No. 1 anaesthetized with thiopental sodium (13.5 min.)	34
26	Sternarchus albifrons No. 1 anaesthetized with thiopental sodium (37.5 min.)	34
27	Sternarchus albifrons No. 2 in aquarium water	36
28	Sternarchus albifrons No. 2 in MS-222, 10 minutes	36
29	Set-up for recording	38
30	Close-up of the set-up for recording;	38
31	Sternarchus albifrons after d-tubocurarine injection	40
32	Ampullary, tonic electroreceptors	40
33	Set-up for recording	41
34	Anaesthetized, curarized Sternarchus albifrons	41
35	Microelectrode amplifier	43
36	Preamplifier	44
37	Synchronous tonic electroreceptor recording	44
38	Nonsynchronous phasic electroreceptor recording	44
39	Gymnarchus niloticus Cuv. #2 electric activity	44
40	Gymnarchus niloticus Cuv. #3 electric activity	46
41	Gymnarchus niloticus Cuv. #2 electric activity	46
42	Gymnarchus niloticus Cuv. #2 electric activity	46
43	Gymnarchus niloticus Cuv. #3 electric activity	46
44.	Gymnarchus niloticus Cuv. #3 electric activity	47
45	Gymnarchus niloticus Cuv. #3 electric activity	47
46.	Gymnarchus niloticus, Cuv. (baby) - transversal cut	48

Figure		Page
47	Gymnarchus niloticus Cuv. - air bladder and spinal cord	49
48	Gymnarchus niloticus Cuv. - spinal cord	49
49	Gymnarchus niloticus Cuv. - brain	49
50	Delafield - Harris hematoxylin staining method	50
51	Malapterurus electricus	52
52	Malapterus electricus on the scale	53
53	Device for measuring the voltage	53
54	Discharges of the electric organ of Malapterurus electricus	54
55	Tonic, ampullary electroreceptor	56
56	Phasic, tuberous electroreceptor	57
57	Tonic electroreceptor	59
58	Phasic electroreceptor	60
59	Ampullary tonic electroreceptor	61
60	Stimulus recording	61
61	Tuberous phasic electroreceptor	61
62	Stimulus recording	61
63	Electroreceptors of Gymnarchus niloticus	62
64	Underwater pattern recognition system	63
65	Recording from an anaesthetized, curarized Sternarchus albifrons specimen	66
66	Microelectrode recording	66
67	Gymnarchus niloticus - photo	70
68	Gymnarchus niloticus - placement of electric transmitting and receiving organs	71
69	Fish Laboratory A	72
70	Fish Laboratory B	72

Table		
1	Underwater pattern recognition system	55

SUMMARY

The electric organs of Sternarchus albifrons, a South American fresh water weak electric fish, have been studied with emphasis on electroreceptors. The morphological and physiological characteristics of electroreceptors, ampullary and tuberous, are discussed. Special instrumentation required for the role of these electroreceptors in pattern recognition has been developed.

We have recorded with microelectrodes the autonomous autorhythmic electrical activity of the tonic asynchronous ampullary electroreceptors of the South American weak fresh water electric fish Sternarchus albifrons. We have also recorded the electrical activity from the phasic tuberous electroreceptors and of the synchronous ampullary electroreceptors of the same electric fish, Sternarchus albifrons. Preliminary measurements have been made. The electric discharge of Malapterurus electricus, an African fresh water strong electric fish, has been measured in and out of water. The autorhythmic activity of the ampullary electroreceptors has been demonstrated.

We obtained some specimens of the African weak fresh water electric fish Gymnarchus niloticus. They are supposed to be the most sensitive of all the weak electric fishes known. Together with two specimens about one foot long, we received a number of baby Gymnarchus niloticus about two inches long. The baby electric fish were infected with a Saprolegnia fungus and could not be saved, but we fixed a number of them in buffered formaldehyde and one of them has been cut and mounted in paraffin for histological studies of the electric organs. Preliminary measurements have been made on the communication capability of adult Gymnarchus niloticus.

A study of the anesthetizing effect of tricaine-methanesulfonate (MS222 = FINQUEL) on Sternarchus albifrons has been undertaken by plotting time for the anesthesia and recovery for different specimens.

A study of an anaesthetic which does not affect the electric fish's electric organ pulse repetition rate is presented.

Also, the effect of D-tubocurarine and the counter-effect of neostigmine has been assessed for Sternarchus albifrons. Finally, some improvements in the micro-electrode recording instrumentation have been made.

The electric organs of Sternarchus albifrons, a South American weak fresh water electric fish, have been studied with emphasis on electroreceptors. Recordings have been made from the asynchronous tonic as well as the synchronous tonic and the asynchronous phasic electroreceptors. The electroreceptors are part of the complex lateralis line system of the electric fishes.

The other lateralis line system sensory receptors, like mechanical receptors and displacement receptors, have been discussed as part of a general hybrid pattern recognition system of the fish. A passive hybrid underwater pattern recognition simulation system has been advanced.

A simulating model concept could be established for underwater pattern recognition through electric sensory receivers and electric fields. More histological work is needed to establish the relationship between different electroreceptors and their innervation. This is also needed for a realistic simulation system of the underwater pattern recognition ability of the electric fishes.

I. INTRODUCTION

In one of our previous reports, we described the morphology of the electric organ of Sternarchus albifrons, a weak fresh water electric fish from South America.[1] We mentioned that the electric transmitting organ of Sternarchus is derived from nervous tissue and not from modified muscle tissue like the majority of other electric fishes. This is making Sternarchus different from other electric fishes: it has a very high signal rate and the signal is phase and amplitude modulated.

The form, rate, and amplitude of the signals emitted by the electric fishes are as diverse as the forms and sizes of these fishes. Some of the weak signals are used to locate objects or animals in their environment, or for navigation, species recognition, and communication.[2] The strong electric discharges serve for offense or defense. Watanabe and Takeda[3] investigated the effect of a-c current with a frequency close to the electrical signal emitted by Eigenmannia. When the applied pulses came within ± 3 to 4 pps of the one emitted by Eigenmannia, the fish would change its rate by 4 to 5 pps in a direction which increased the pps separation. Increasing the frequency of the applied a-c current in 1 pps increments caused the fish to shift its frequency correspondingly until it reached about 6 to 7 pps over its normal rate, when it would revert to its original rate. We obtained similar results in experiments with Sternarchus albifrons, but the applied a-c signal was within 0.5 cycle of the signal of the fish, demonstrating how specific the applied signal must be to elicit a change in the Sternarchus signal rate: ± 0.5 cycle is the range of selectivity of the fishes' electrical transmitting-receiving system. The fact that many fresh water and sea water electric fishes have never been studied may present some difficulties in obtaining special kinds of electric fishes, and their care may not be an easy task. There are, however, enough species to enable many experiments.

Very little is known about the electrical activity of marine electric fishes except Torpedo and some rays. Narcine Barziliensis is the only known marine electric fish having two different electric organs. Bennett[4] studied the mode of operation of the electric organs of Raja eglantaria, a marine electric fish, and of the fresh water fishes Hypopomus and Sternopygus and compared them with Narcine, Mormyrus, Steatogenys, Gymnorhamphychtis, Malapterurus, Gymnotus carapo, and Electrophorus electricus.

The main objects of this study were the form, innervation, and physiology of the electroplates forming the electric transmitting organs. The electroplates of Hypopomus, Gymnotus carapo, Malapterurus, and some Mormyridae have the same surface area and produce spikes during discharge. The electroplates of Sternopygus and possibly Eigenmannia have two peculiar characteristics: there is a steady potential on which pulses are superimposed, and the resistance of the electroplates is similar at the peak or between the spikes. The electroplates are of the type with a slow depolarization.

In Hypopomus, Malapterurus, and most of the Mormyridae, the innervation of the electric organs is through stalks. The stalks may serve to amplify depolarization until it would be able to invade the body of the electroplates.

The discharge rate and duty cycle have been compared for electric fishes like Sternopygus (rate = 50/sec) and Eigenmannia (rate = 280/sec) with the Sternarchidae (max. 1500/sec). Compared with mammalian central nervous systems, peak frequencies of the electric organs are not greatly different. The Renshaw cell can discharge impulses at a rate of 1400/sec[5], and neurons in the sensory path sometimes produce bursts at a rate of about 1000/sec.[6]

In an examination of discharge pattern and organ function studies, it was noted that Grundfest[7] defined two groups of electric fishes: those that emit signals at a constant

rate, and those that emit pulses with a variable rate. For example, Gymnarchus, Sternopygus, and Eigenmannia are in the constant rate group; Electrophorus, Gnathonemus petersii, Steatogenys, and Hypopomus belong to the variable rate group. Bennett[4] did not make any connection between the electric fishes' electrical systems, their environment, and their behavior. No one investigated their evolution, very little is known about their mating or birthplace, and no one has reported the breeding of electric fishes confined in water tanks.

Sternarchus albifrons also has two kinds of electroreceptors: tonic and phasic, and they are autorhythmic. These electroreceptors are sensitive to movement and direction. The phasic electroreceptors seem to be related to information regarding movement of objects near or around the fish.

Accepting the principle of pacemaker activity in the brain, it seems that there are only a reduced number of command nuclei acting on the electric transmitting organ. It is also reasonable to assume that electrically mediated positive feedback must be present; chemically mediated transmission would be too slow for the repetition rate of transmission which can attain under certain circumstances over 1,300.

Mauthner cells of lower vertebrates (Figure 1) can be considered single cells command system for the axial musculature on either side of the body[8,9]. In the hacketfish each Mauthner fiber activates the muscles depressings both pectoral fins and these cells thus constitute a bilateral command system for the depressor muscles.[10,11] For explaining the pacemakers action of the command nuclei in the brain of the mormyrid electric fishes a mutual excitation with positive feedback has been proposed. This theory would not work for the Mauthner cells. There is a crossed inhibition between the Mauthner cells in the brain of the goldfish and it could equally be effective in an electric organ system. There is a requirement of high speed of transmission in synchronized systems like many of the electric transmitting organs. This has been useful in predicting sites where transmission has been

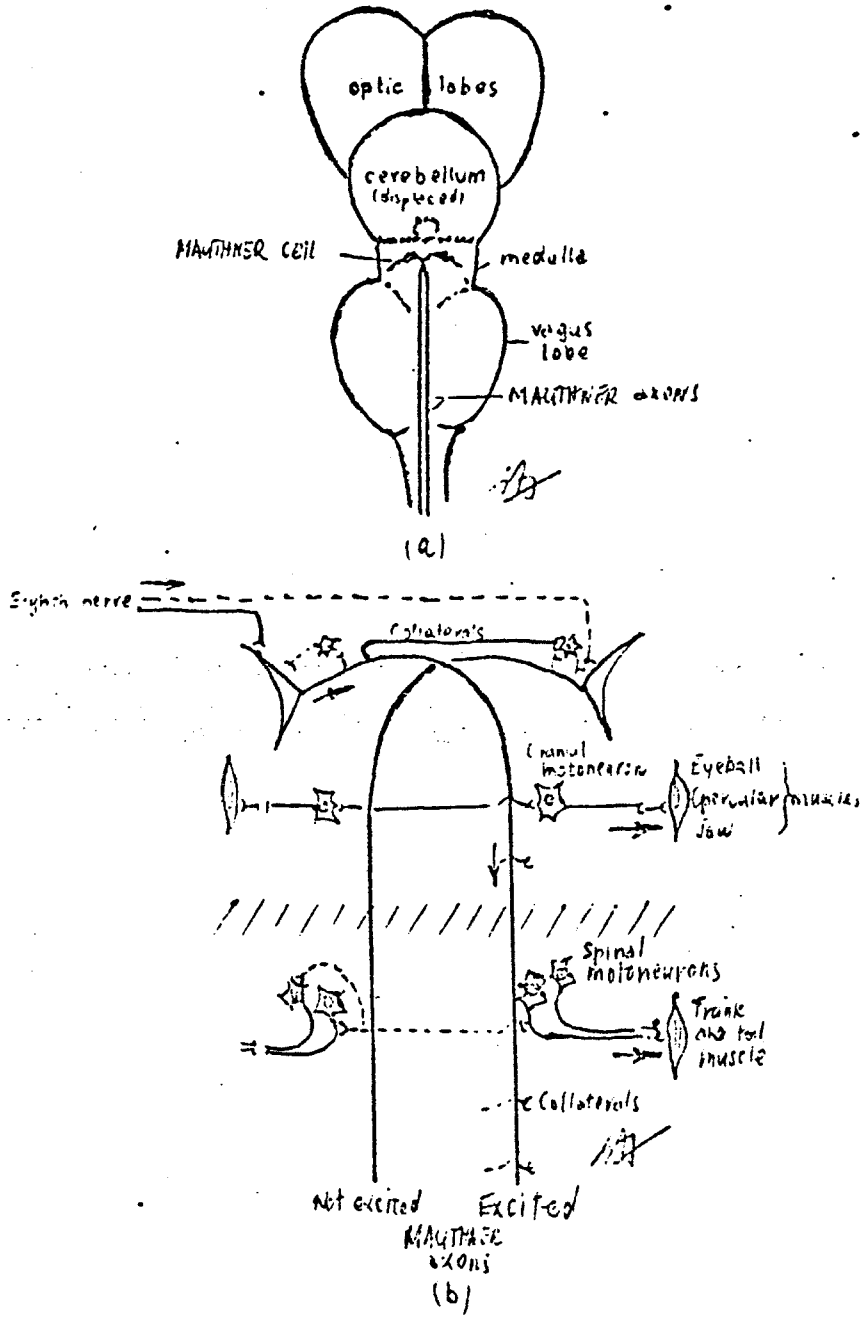


Figure 1. (a) Schematic drawings of the location of the Mauthner cells (from Furukawa and Furshpan[2])

(b) The principal circuitry involved in Mauthner reflex (only the VIII-th nerve connections and collaterals deriving from the excited Mauthner cell are shown).

electrically mediated. Positive feedback may be a "sine qua non" requirement for the pacemaker nuclei of electric transmitting organs. There is proof of positive feedback in the mutual inhibition system of arthropod compound eyes[12,13].

The neural systems controlling electric organs have provided a large number of examples of electrically mediated transmission, which meets the functional requirement for rapid communication between cells. This mode of transmission also proves to be able to mediate many functions often considered as restricted to chemically mediated transmission. The correlation between morphologically close apposition and electrotonic coupling was considerably strengthened by the work on electromotor systems. This correlation helps to validate morphological identification of electrical transmission in other systems where electrophysiological analysis is not so simple.

It is not known whether there is any relevance to higher systems of the organizational principles deduced from electric organ systems. The next level of analysis of the electric organ systems may be no easier than the study of less specialized systems that are of more general interest. Some knowledge is being obtained of afferent pathways from electroreceptors in weakly electric fish which have important inputs to the electric organ control system. Both operant and respondent conditioning of the control system can be obtained and conditioned response latency can be very short. It is not unreasonable that the complete neural pathway of the conditioned response could be obtained in these cases. The central connections are still minimally explored; one knows what goes in and one can go from the electric organ several synapses antidromically. The rewards for filling in the gap would be great, and prospects for at least some progress are bright.

In Sternarchus albifrons electroreceptors are distributed over the entire body. The phasic tuberous receptors are very numerous as compared with the tonic ampullary receptors (Figure 2). The density of receptors is greatest in the head region and

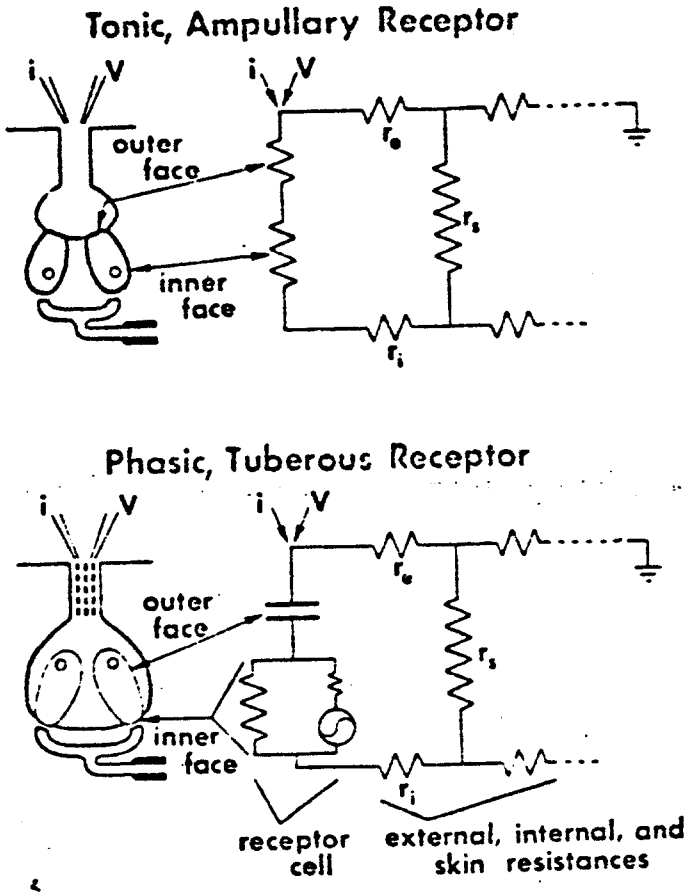


Figure 2. Anatomical diagrams and equivalent circuits of electroreceptors in fresh water electric fish: (a) tonic, ampullary receptor and (b) phasic, tuberous receptor. Diagrams are shown with the external medium to the top. The skin and wall of receptor cavities are shown in cross section as lines. The opening to the exterior of phasic receptor cavity is shown as occluded by a porous mass. The nerve fibers innervating receptor cells are indicated.

falls gradually toward the posterior end. There are minor morphological subdivisions within the phasic and the tonic receptors, but no physiological correlations have been yet obtained[14,15,16].

In Figure 2 the equivalent circuits of tonic, ampullary and phasic, tuberous organs is shown. Cross sections through the receptors are shown with the external medium to the top. The skin and walls of the receptor cavities are represented by lines, innervation of the receptor cells is indicated.

The electroreceptors over the entire body are innervated by the anterior lateral line nerves, a large branch of which runs posteriorly to join the posterior lateral line nerve just behind the head[17] (Figure 3). The posterior lateral line contains only mechanoreceptive fibers which come from free neuromasts and canal organs[18,19].

The receptor cells of tonic receptors appear to behave very nearly like linear elements; that is, their membranes have fixed internal potentials, resistances, and capacitances. They are, in a sense, electrically inexcitable, and they differ markedly in this respect from phasic receptor cells. There is evidence for chemically mediated transmissions at tonic receptors of gymnotids[20]. The morphological characteristics of the snyapse are those typical of chemically mediated transmission. A strong brief anodal stimulus produces an evoked response long outlasting the stimulus. Then there is a synaptic delay between the initiation of the impulse and the nerve impulse (between 0.5 and 1.5 msec). Mormyrid tonic receptors are similar to those of gymnotids and Gymnarchus niloticus tonic receptors are morphologically similar, but they were not physiologically studied[21].

The relationship between the different electroreceptors of Sternarchus albifrons in pattern recognition has not as yet been studied. The present study developed special instrumentation required for investigating the roles of these electroreceptors in pattern recognition and obtained preliminary measurements of electrical discharges from Malapterurus electricus in and out of water.

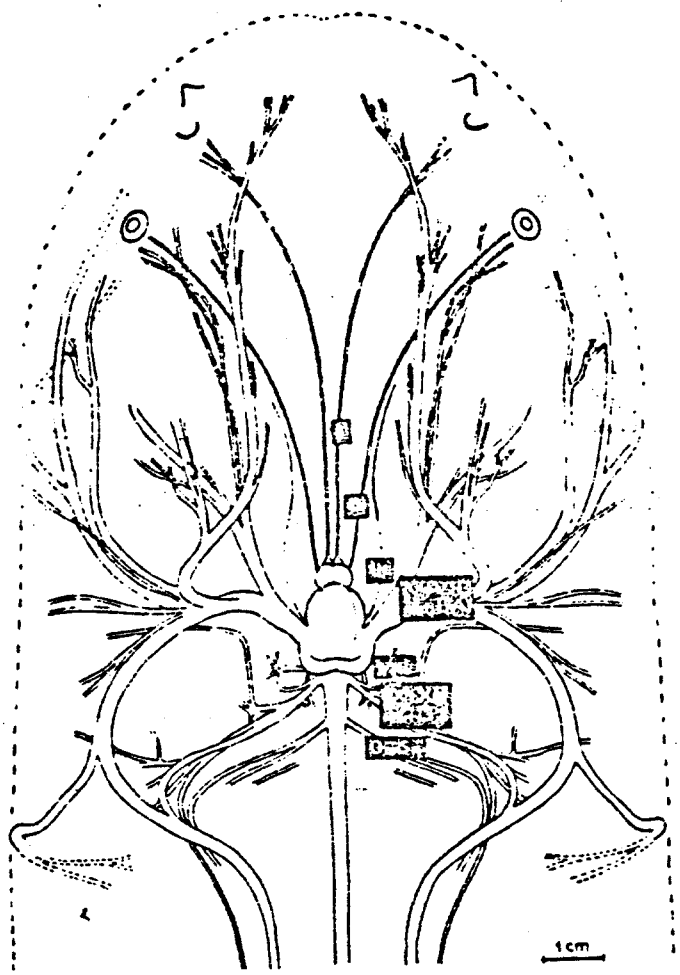


Figure 3. Horizontal projection of the cranial nerves of Electrophorus. Note the small size of the brain. Some nerves are indicated by the corresponding numbers. L.A. nervus lateralis anterior; LP nervus lateralis posterior; Oc-Sp, occipito-spinalis nerve.

In our experiments, the perception of objects by weak electric fishes by way of the discharge of their electric organs (transmitting and receiving) has been demonstrated. Other authors[22] have shown that weak electric fishes can be trained to distinguish between conducting and nonconducting objects placed in the water.

For this kind of reception, two possible modes of action at the level of specific receptors could be proposed: the "pulse-frequency-modulation"[22] and the "pulse-phase-modulation"[23]. According to the first hypothesis, sensory information should be conveyed by the frequency of the sensory impulses dependent on the pulse of the electric organ discharge whereas, according to the second hypothesis, the time relation (the phase) between the electric discharge and the following sensory impulse would play an important role for the sensory coding. From our experiments with Sternarchus albifrons, a South American weak fresh water electric fish, we concluded that apparently neither of the above proposed mechanisms are operating in this fish. The intensity of current flowing at the level of the receptor is coded by the number of impulses elicited by each electric organ discharge. Sternarchus albifrons can discharge at rates higher than 1000 per second and the sensory impulses of the receptors can follow their discharge rate.

In some mormyrids[24] and gymnotids[25], however, modulation of the relation electric-organ pulse-sensory impulse may be used for electrosensory coding. In mormyrids, also, the change in intensity of the electric field may be coded by changing the latency between the electric organ pulse and the sensory receptors' impulse. In mormyrids, it may attain values up to 9 msec, whereas in gymnotids it has been found not to exceed 1-2 msec.

It has been previously mentioned that in Sternarchus albifrons the electroreceptors are distributed over the entire body of the fish and that the ampullary tonic receptors are more numerous than the tuberous phasic receptors. We recorded then the autorhythmic electrical activity of the nonsynchronous tonic, ampullary electroreceptors of Sternarchus albifrons. The impulses were irregular around a repetition rate of

between 100 and 300, with an amplitude of around 2.5 mV. The impulse duration was around 200 microseconds. There are other types of tonic receptors which are synchronous. The phasic units are nonsynchronous.

The nonsynchronous tonic receptors seem to react independently from the transmitting electric organ. They react to any objects brought near the fish at a certain range. The recording shown in our final report is made from such type of receptors.

As previously mentioned, some fresh water weak electric fishes have the ability of perceiving objects, their movement and direction, and also to determine some characteristics of these objects (such as conductivity). For this underwater pattern recognition, they utilize both their electric transmitting organs and their electroreceivers. Obviously some other sensory perception receivers of the lateralis line may be involved such as: free neuromasts and the cupula type of lateral line receivers; the auditive system may play a role also in this pattern recognition. Some of the electric fishes are blind or have vestigial eyes; others have good vision and it is certainly used in the recognition process.

It seems that a hybrid system made of a diversity of sensory receptors is used by the electric fishes to locate and identify objects and fishes of the same or different species and also prey or predators. They use little electrical energy for this and the distance involved is considerable — it may attain, under certain circumstances and for one specific sensitive species (Gymnarchidae), over a mile.

The strong electric fishes (Electrophorus, Malapterurus, Torpedo, and Astroscopus) can incapacitate and kill their prey with their discharge of the powerful electric organ used only for such purposes. If they have a detecting and locating system, they use a different low power electric transmitting than the main powerful electric organ (i.e., Electrophorus). The meaning of this is that there is no high power requirement for the pattern recognition system of electric fishes. The electric

sensory receptors are very sensitive, have a high discriminating capacity, and use an electronic processing system based on a multiple degree of freedom modulating and coding system; they are also jamming resistant.

The importance of such an underwater pattern recognition system cannot be overemphasized. The simulation and modeling of it can be achieved once the parameters of the different sensory receivers have been established, physical analogs derived, and models devised.

By and large, the communication and coding system of electric fishes has been established and discussed[26]. The antijamming capability of the system has been demonstrated through simulation[27]. The electroreceptors of Sternarchus albifrons, a fresh water weak electric fish, have been studied, and for two different kinds, physical analogs have been proposed.

Because of our previous findings that the anaesthetic "MS 222 (Finquel)" used by most researchers of electric fishes affects the frequency of the impulses emitted by the electric organs in a nonuniform way, we investigated a number of different anaesthetics and found one which does not affect the frequency of the impulses or their amplitude.

II. INSTRUMENTATION

The recordings of the autorhytmic electric signals from electroreceptors need special instrumentation. The signals are of low voltages: less than 1 mv, and they need a large bandwidth. The diameters of some of the electroreceptors are of the order of microns. There is the need for an insulated microelectrode with a tip of one micron, with a reasonable low resistance, possibly less than one megohm, responding from DC to several kHz with low distortion and enough rugged to withstand some bumping by the fish. It has also to be of a nonpolarizable type. We developed a microelectrode with all these characteristics and it is described later in this chapter.

In our proposal for the continuation of the investigation of electric fish we mentioned that we developed a sensitive low noise solid-state microelectrode d-c amplifier (Figures 4, 5). We modified them having now a Motorola MC 1531 input stage and a Motorola MC 1431 output stage. Graphs show the repetition rate versus amplification factor for square wave and frequency versus amplification factor for sinusoidal waveforms with a 100 kiloohms, 1 megohms, and 5 megohms input resistance (Figures 6, 7).

We give a description of the method used to produce a suitable microelectrode for recording electric signals from the electroreceptors of Sternarchus albifrons.

Hubel[28] described how to make coated tungsten microelectrodes. Wohlbarsht, et al,[29] described glass insulated platinum microelectrodes; Green[10] and Grundfest, et al[31], used stainless steel electrodes. The steel and tungsten electrodes have a fairly high resistance (20 to 100 megohms) and all, including the platinum electrode, are polarizable. Donaldson[32] describes a multitude of microelectrodes such as silver-silver chloride, platinum-platinum chloride, and others. Silver-silver chlorides are very convenient

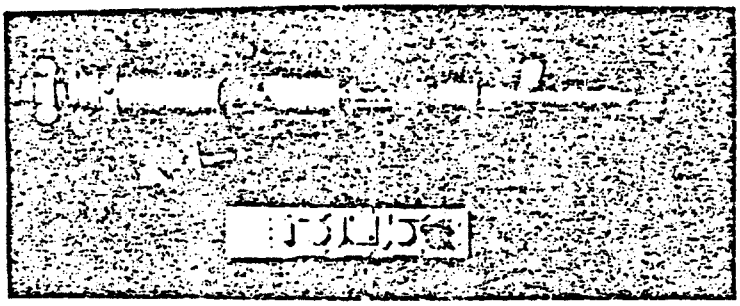


Figure 4. Microelectrode amplifier with one MC 1531 and one MC 1431 operational amplifiers.

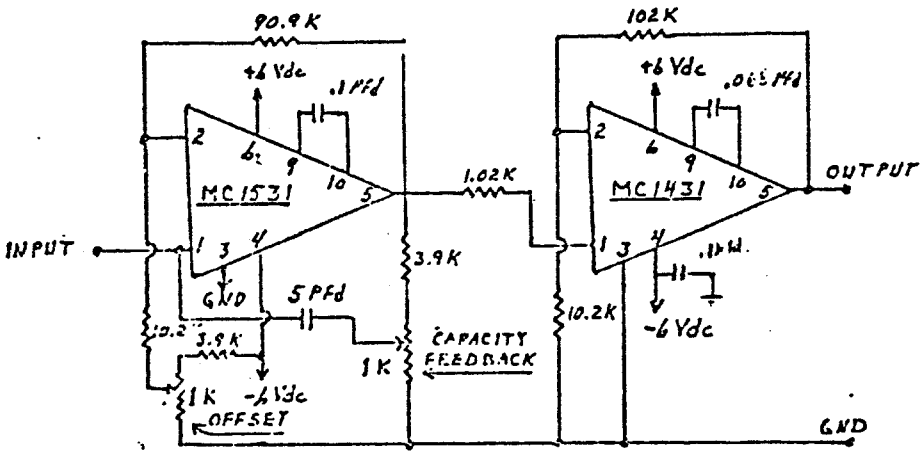
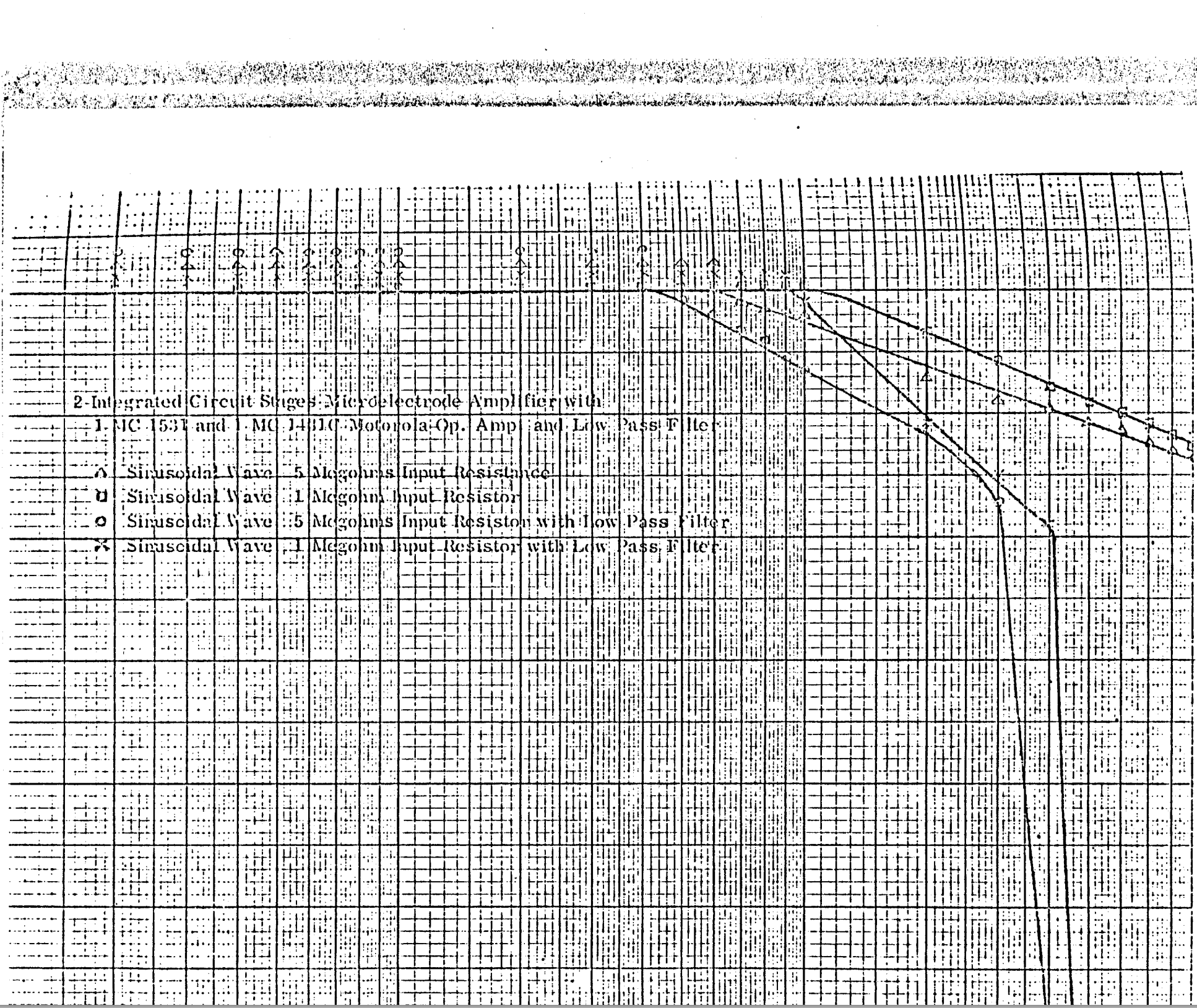
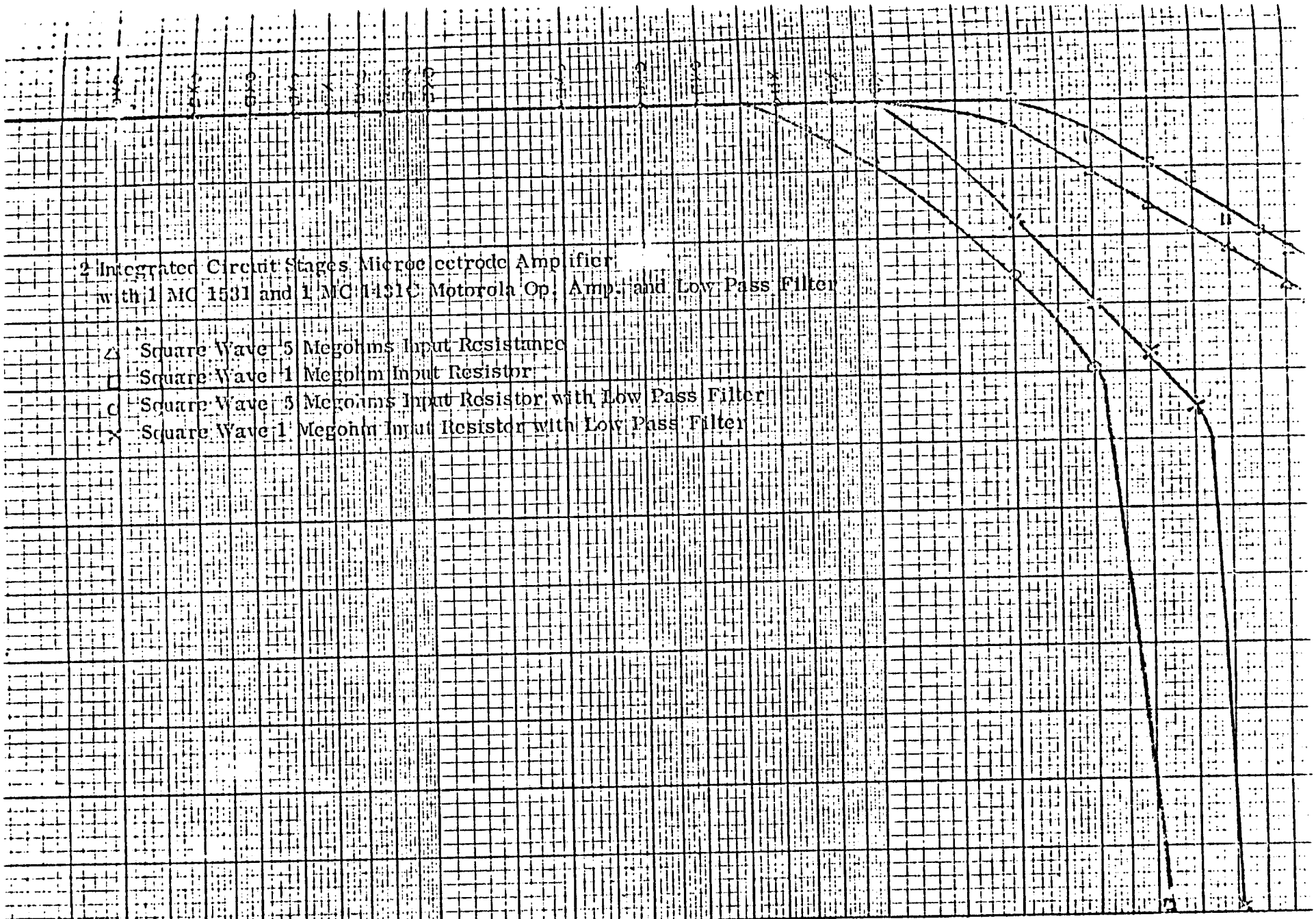


Figure 5. Microelectrode amplifier with one MC 1531 and one MC 1431 operational amplifiers: schematic diagram.


2-Integrated Circuit Stages Microelectrode Amplifier with
1 MC 1531 and 1 MC 1401G Motorola Op. Amp and Low Pass Filter
Sinusoidal Wave .5 Megohms Input Resistance
Sinusoidal Wave .1 Megohm Input Resistor
Sinusoidal Wave .5 Megohms Input Resistor with Low Pass Filter
Sinusoidal Wave .1 Megohm Input Resistor with Low Pass Filter


2 Integrated Circuit Stages Microelectrode Amplifier
with 1 MC 1531 and 1 MC 1510 Motorola Op. Amp. and Low Pass Filter
Square Wave 5 Megohms Input Resistance
Square Wave 1 Megohm Input Resistor
Square Wave 5 Megohms Input Resistor with Low Pass Filter
Square Wave 1 Megohm Input Resistor with Low Pass Filter

7. LOSS IN -dB WITH INCREASING REPETITION RATE OF SQUARE WAVES WITH AND WITHOUT LOW PASS FILTER

electrodes but have a high resistance. In order to lower the resistance, it is advisable to cover the silver-silver chloride electrode after the electrode is insulated with insulex lacquer, with platinum black through an electrolytic process and after this to add a new silver-chloride coating. The tip of the electrode can be anywhere between 0.5 and 1 micron in diameter.

Take a 50 ml beaker and fill it with conc. H Cl and cover it with Xylene (about 1/2"). Use a carbon rod for one electrode (spectroscopy carbon rods are suitable) and connect it to a variable source of A.C. current from a variac and a bell-type transformer (app. 7 to 12 v). Connect the low voltage leads to an A.C. voltmeter. Use about 5 to 6 volts to sharpen the silver wire #36 gauge (any necessary length) by moving it up and down for about one minute (Figure 8). Decrease the voltage to between 2-3 volts and move the electrode rapidly for 30 seconds up and down. Have 50 ml beakers filled with: (1) saturated sodium carbonate solution (Na_2CO_3), (2) acetic acid 1% in H_2O $(CH_3CO)_3$ - O, (3) Ethyl-alcohol 200 proof, (4) Xylene. Move electrode after sharpening process from No. 1 through No. 2, 3, and 4, agitating a few times the electrode in the liquid. Check under microscope with a microfilar for sharpness; if not sharp enough, operation II (2-3 volts) and the cleaning from 1 to 4 should be repeated.

If sharp enough, take a 50 ml beaker and fill it with Na Cl 1% solution in distilled H_2O. Use a silver wire (No. 18 to 22 gauge) as an electrode (cathode) and connect it with a D.C. source (power supply) of between 1 to 2 volts. The positive end should be connected to the microelectrode. Hold it for 30 seconds in the H Cl solution. Reverse twice the polarity for the same amount of time (Figure 9). Wash the electrode in distilled water for two minutes. Insulate the electrode with insulex lacquer. For drying, set the microelectrodes with the tip up. Dry for 24 hours. The tip should be clean of lacquer for 10 to 30 microns. Take a 50 ml beaker and fill it with a 1% chloroplatinic acid. Use a No. 18 or 22 gauge wire as a cathode

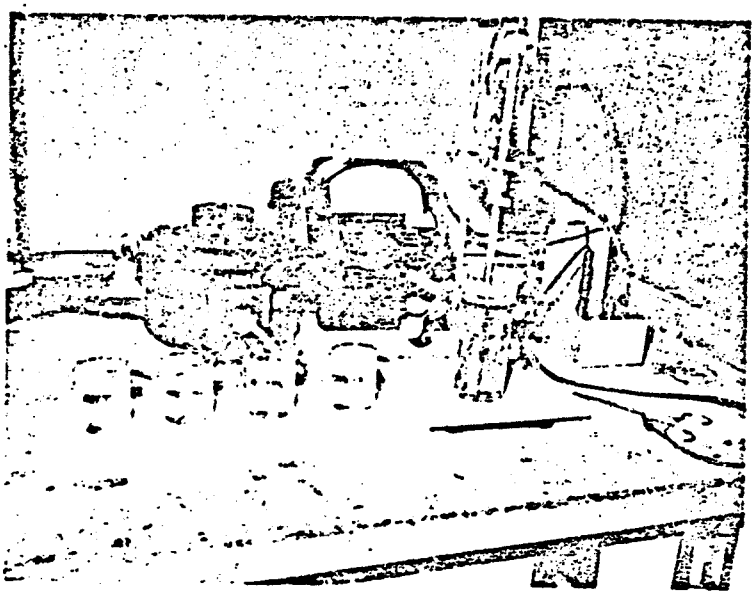


Figure 8. Device for sharpening metal electrodes.

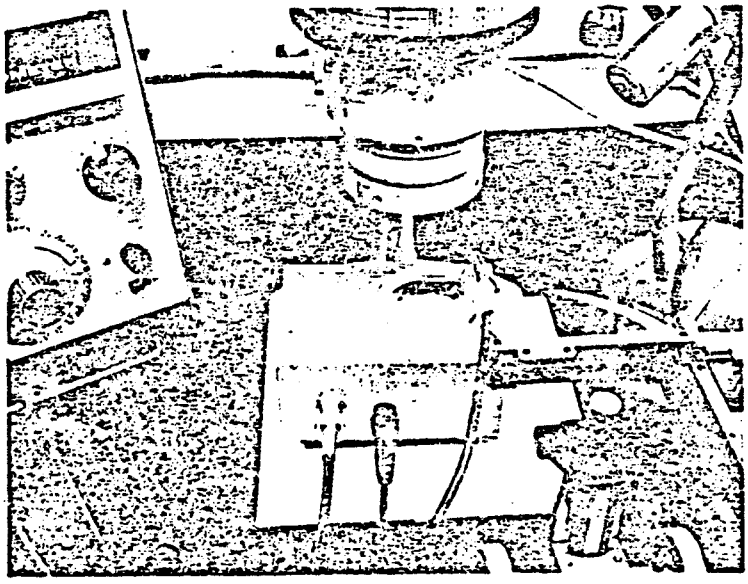


Figure 9. Device for applying silver chloride on the tip and surface of silver or silver chloride platinized electrodes.

connected (to the negative) to a D.C. source of 15 volts. The microelectrode should be connected to an anode (positive) of 15 volts, in series with a 1 Megohm (1 W) resistor. Pass current through the electrodes for 15 to 30 seconds. Wash the electrode in distilled water for a few minutes. Use the 1% Na Cl solution with a silver wire, gauge No. 18 to 22, as cathode, and the 15 volts D.C. source in series with the 1 Megohm resistor for depositing a silver-chloride coating on the tip of the microelectrode. Twenty to 30 seconds will be sufficient. If bubbles come off from any other part than the tip, it means the insulation is not good and should be redone. Wash the electrode in distilled water for 10 to 15 minutes. Store it in dark container filled with Ringer solution.

The electrode has low resistance (from 100k to 800k depending on the tip), is non-polarizable and produces very little distortion from D.C. to fairly high frequency (over 1000 Hz) (Figures 10, 11).

In Figures 11A, 11B, 11C, and 11D, the performance of the microelectrode amplifiers is shown.

Figure 10. Potentiometric device and resistance substitution and series resistance box for measuring micro-electrode resistance.

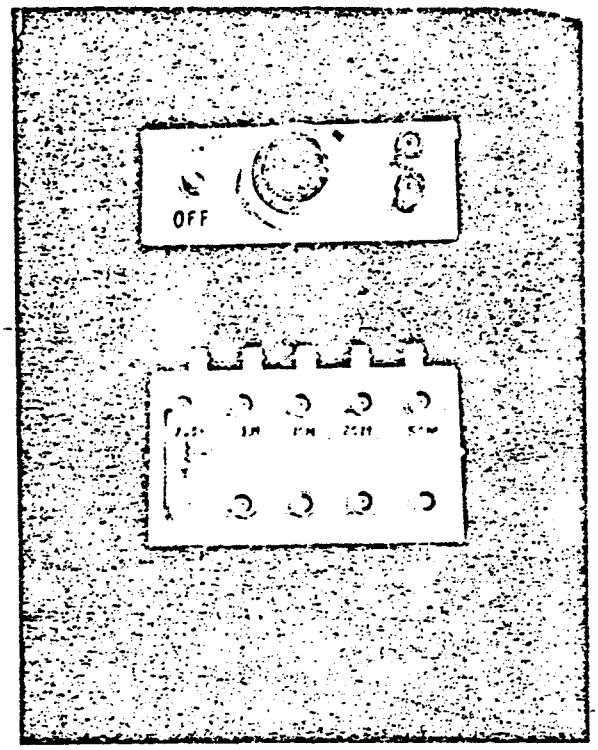
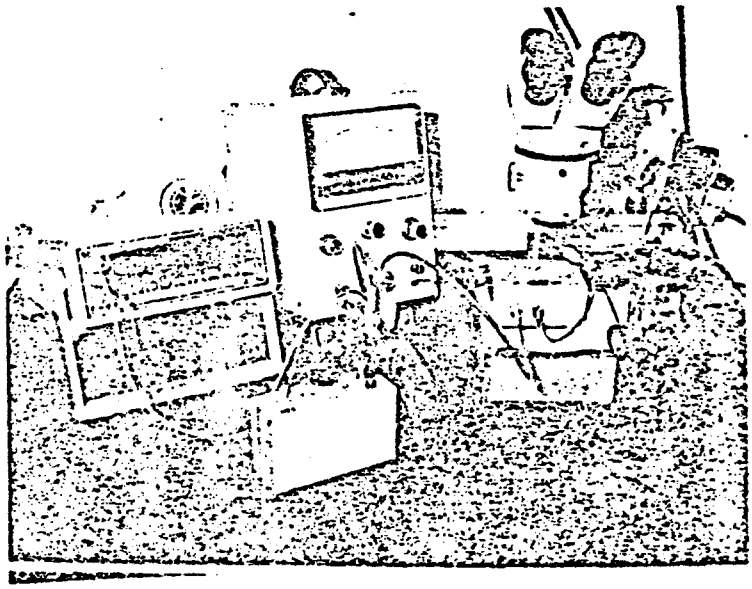


Figure 11. Device for measuring micro-electrode resistance with the aid of the potentiometric device.



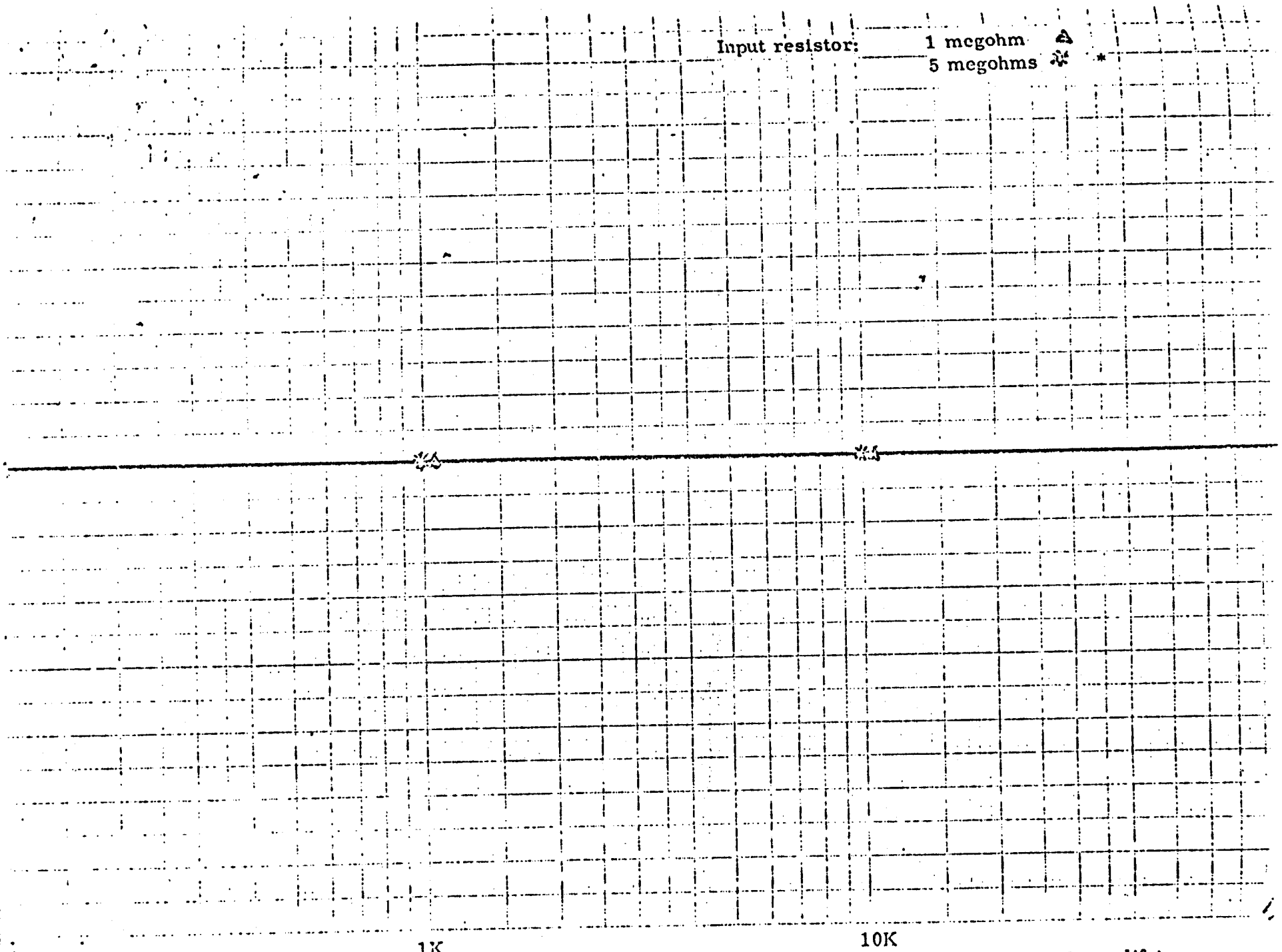


Figure 11A. Amplification factor versus repetition rate of square wave. (2 stage MC 1531 amplif.)

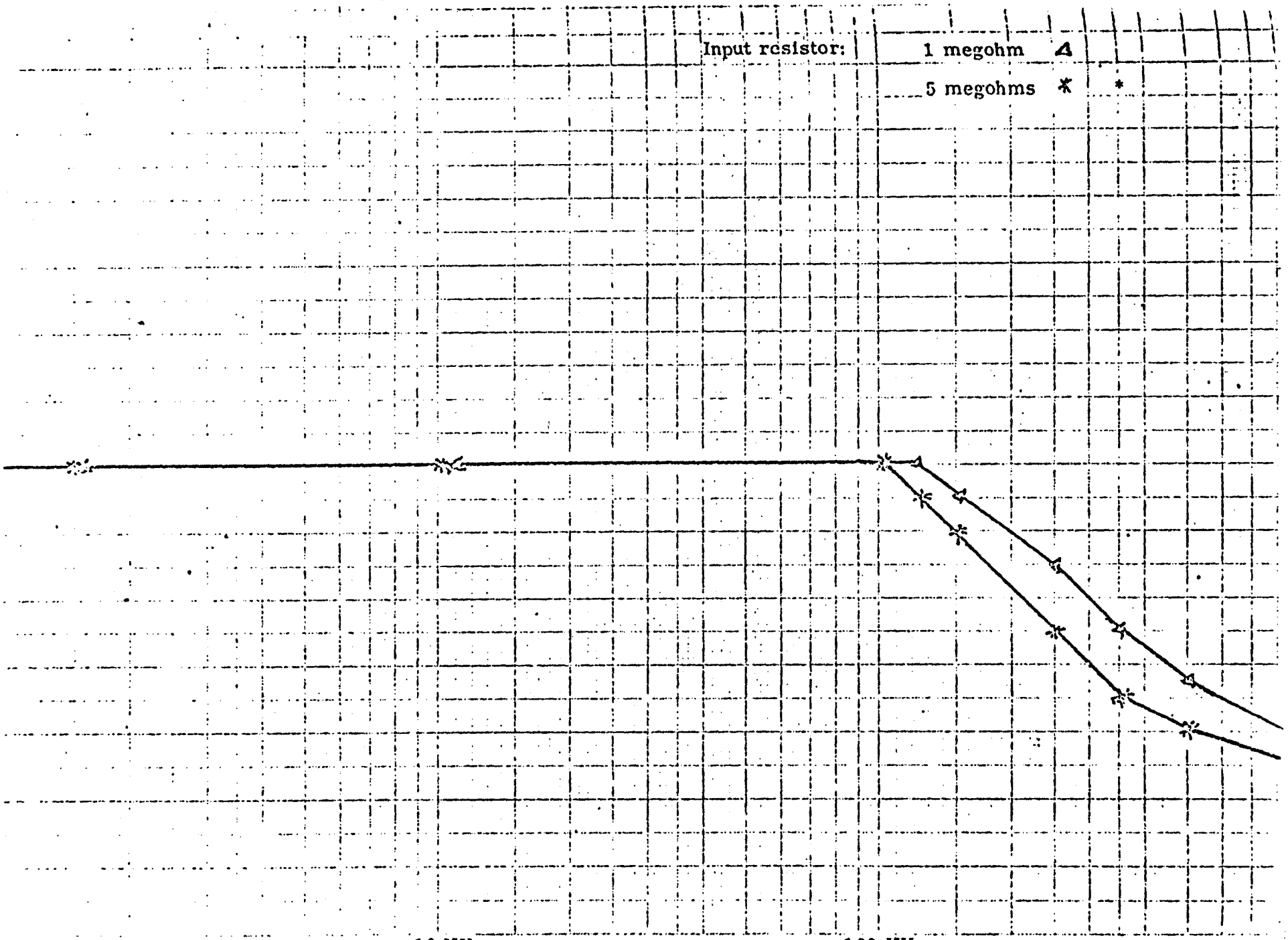


Figure 11B. Amplification factor versus frequency: sinusoidal wave. (2 stage MC 1531 amplif.)

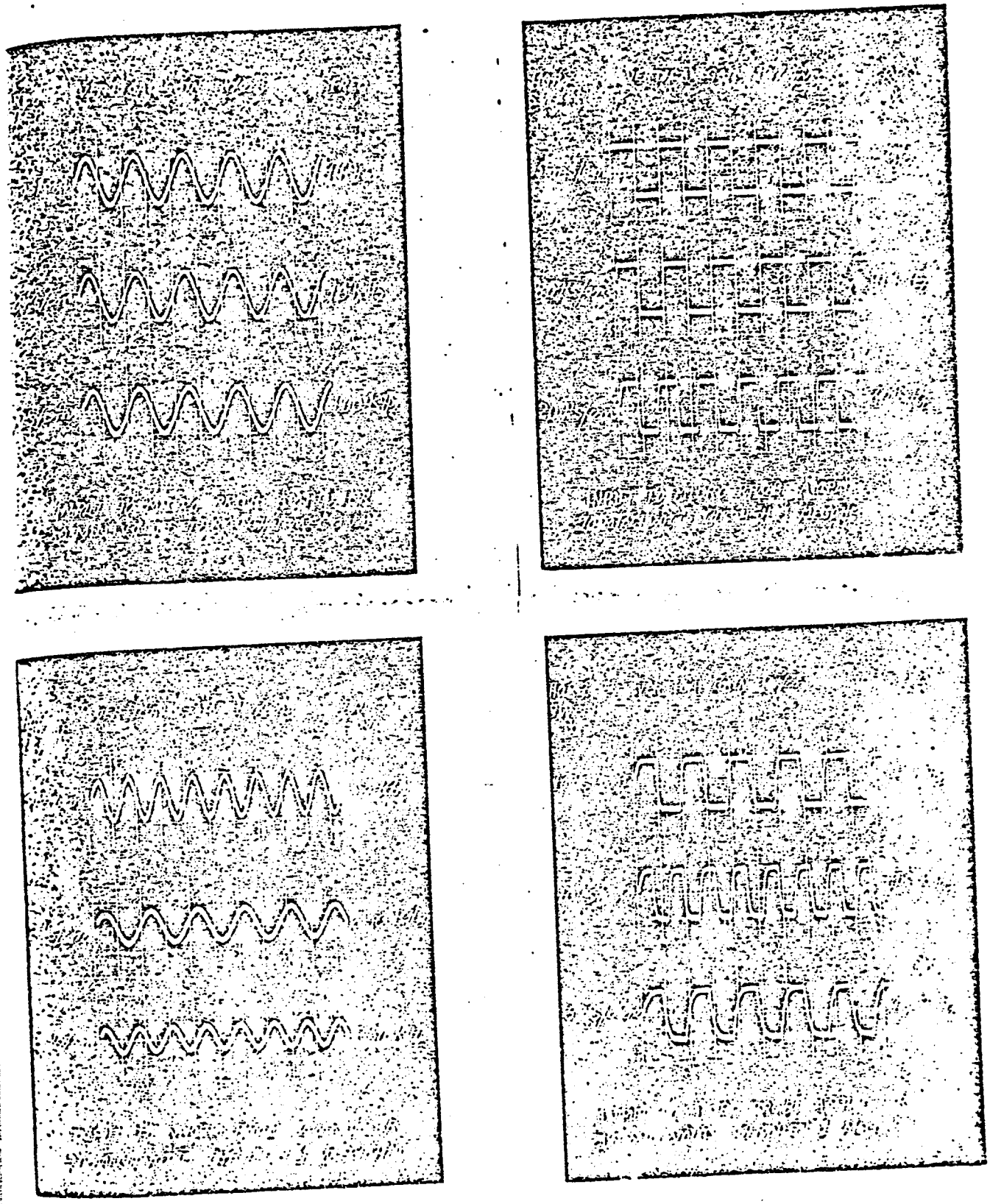


Fig. 11-C Recordings at different square wave repetition rates and sinusoidal frequencies with the double stage MC 1531 microelectrode amplifier input resistance 1 megohm.

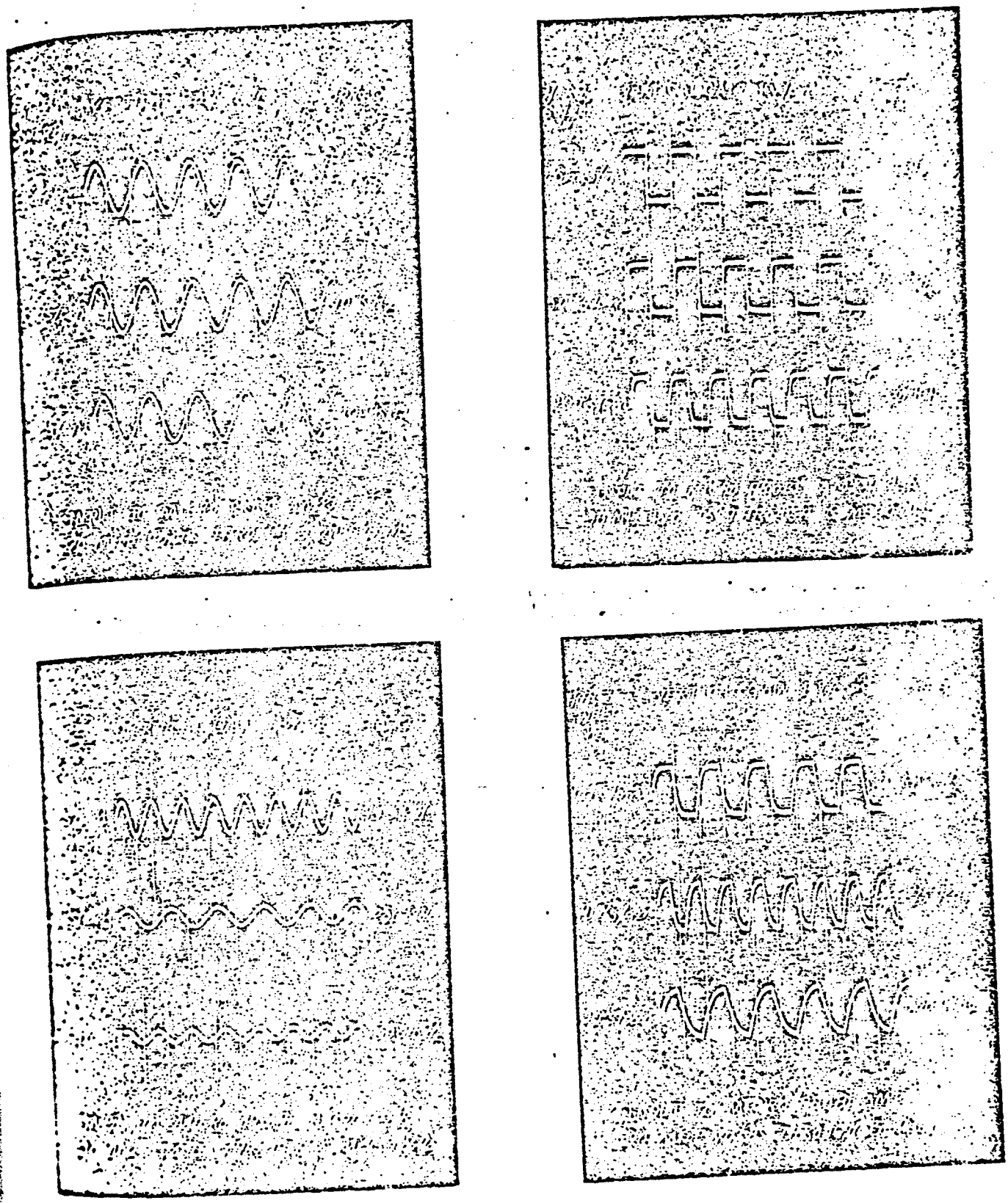


Fig.11-D Recordings at different square wave repetition rates and sinusoidal frequencies with the double stage MC 1531 microelectrode amplifier input resistance 5 megohms.

III. A SEARCH FOR AN ELECTRIC FISH ANAESTHETIC THAT WOULD NOT AFFECT THEIR ELECTRIC SIGNAL

A commonly used anaesthetic for fishes and cold-blooded animals, urethan (ethyl carbamate = $C_3H_7NO_2$) has been found to have carcinogenic properties by Wood[33] and Ball and Cowen[34]. An editorial comment accompanying Wood's report indicated that the substance named MS-222 (today called Finquel) might be a suitable substitute for urethan. MS-222 (tricaine methanesulfonate = $C_{10}H_{15}NO_5S$) was discovered by M. Sandoz during his search for a reliable synthetic substitute for cocaine (benzoil-methylecgonine = $C_{11}H_{21}NO_4$) for a local anaesthetic.[35]

Since Wood's and Ball and Cowen's reports, MS-222 has become routine for anaesthetization of fishes to facilitate the handling of both marine and fresh water species. The toxicity of the drug to some species of fish was determined by Marking[36]. Walker and Schoettger[37] measured its residues in various tissues of salmonides and its efficacity has been investigated by Schoettger and Julin[38]. The chemical and anaesthetic qualities of MS-222 are mentioned by Klontz[39], and details about the chemistry can be found in the Merck Index[40].

In the last years (1970-71), an agreement has been made between the Sandoz Pharmaceuticals, Division of Sandoz, Inc., Basel, Switzerland, and Hanover, N.J., USA, and the Ayerst Laboratories, New York, N.Y., that the latter should produce and sell the MS-222 (tricaine methanesulfonate) in the United States under their own brand name of "FINQUEL".

In most of the studies on electric fishes, MS-222 has been used as a general anaesthetic by the investigators. Szabo[41], Hagiwara, Szabo and Enger[42], Enger and Szabo[43], Nobuo Suga[44], and many others used MS-222 to anaesthetize electric fishes during surgical procedures and subsequently to record the electrical activity either from the electroreceptors or from the nerves connecting them with the central

nervous system. No mention has been made of the effects of the MS-222 on the frequency and amplitude of the electrical signals as related to concentration and/or time. Bullock[45] mentioned that Hypopomus occidentalis, a South American fresh water weak electric fish, with a normal repetition rate range of 25 to 90 per second, would lower its frequency to below 16 per second only under anaesthesia. Under deep anaesthesia, the fish may stop abruptly its electrical activity. By reducing the level of anaesthesia or by stopping it to let the fish recover, it will also abruptly resume the normal electric activity. In our experiments with Sternarchus albifrons, a South American fresh water weak electric fish, we found a gradual tapering of the repetition rate of the electric signal with the deepness of the anaesthesia and a gradual increase of the repetition rate with the recovery. By monitoring the effect of Finquel (MS-222) anaesthetic on the electrical activity of Sternarchus albifrons, it has been observed a fast and significant change in the repetition rate of the electric signals. A decision was made to study the effect of different anaesthetics on the electrical activity of different species of electric fishes.

The anaesthetic agents of choice were:

1. MS-222 = FINQUEL = TRICAINE METHANESULFONATE
2. NEMBUTAL
3. AMYTAL
4. SECONAL
5. THIOPENTAL SODIUM
6. NOVOCAINE
7. TERTIARY AMYL ALCOHOL

A. INSTRUMENTATION, MATERIALS, AND METHODS

A specially built 30 cm tray made of lucite which could be adjusted to the size of the fish has been used to check the effects of anaesthetics on the electric organs of electric fish (Figure 12). The tray is provided with fittings for the rapid discharge of

anaesthetics or water and a constant aeration of the solution is possible if necessary. It also has at every centimeter distance embedded stainless steel electrodes connected on both sides of the tray with the exterior and to contacts. The electrodes corresponding to the position of the head and tail of the fish were connected through a high-gain, low noise amplifier to two oscilloscopes Tektronix (one for photo-taking), a counter and an FM tape recorder (Figure 13). The tray with the fish was located in a floating screen room and all the instrumentation grounded to the screen room was located in the laboratory outside the screened room. A lucite cover on the tray prevented the fish from jumping out, different holes served to pour in water or anaesthetics, to put in thermometers, and to let excess air out. A d-c (battery) operated high intensity lamp was used to illuminate from the top the fish and to furnish enough heat to hold the temperature constant during experiments. For the experiments, the fishes' own aquarium-water was used at the beginning of the experiment and the same water was used for mixing in the anaesthetics. In this way, the temperature of the water and anaesthetic solution was easy to keep to the same level as the normal temperature of the water in the aquarium. The pH of the aquarium water at the beginning of the experiments and also the pH of the anaesthetic solution were measured with an expanded precision type of pH meter. The pH meter has been calibrated before each experiment.

Tentative measurements and observation of the action of the anaesthetic have been made first on goldfish and then on one of the electric fishes before another specimen has been selected for the experiment. For each experiment with one and the same anaesthetic, five specimens of Sternarchus albifrons have been used. The specimens of Sternarchus albifrons, a fresh water South American sternarchid weak electric fish, have been in our laboratory for over one year and they were all healthy and varied in weight from 14 to 30 grams. Gymnarchus niloticus, a fresh water African gymnarchid weak electric fish, has been kept for over six months in our laboratory. There is another specimen just received. There are also two Gnathonemus petersii, fresh water African mormyrid electric fish, being in the laboratory for over two months.

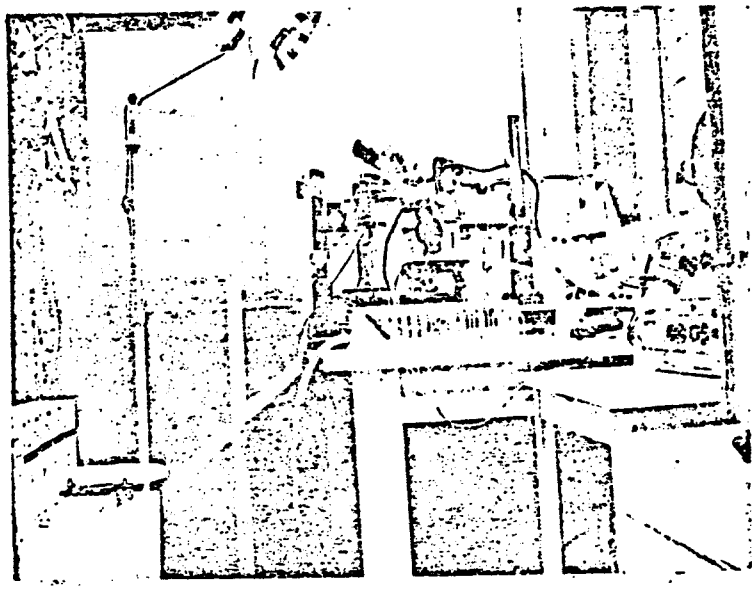


Figure 12. Adjustable Lucite Tray for Anaesthesia Experiments.

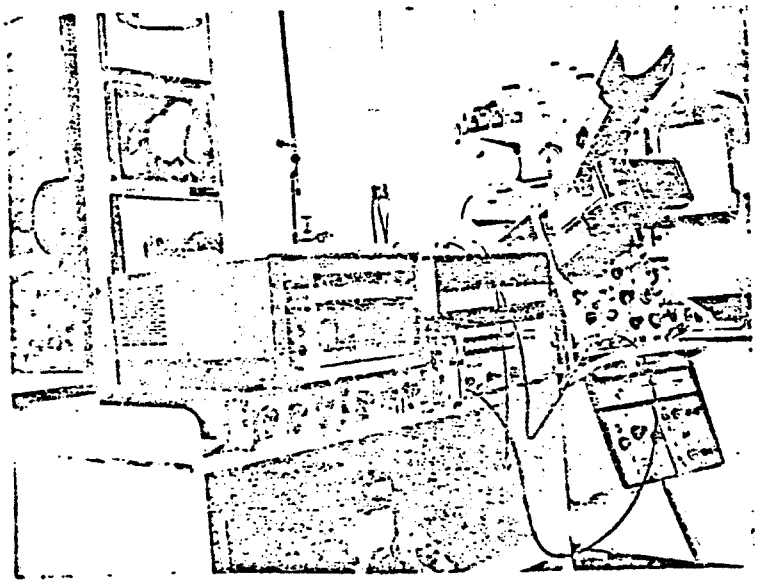


Figure 13. Instrumentation Used for Anaesthesia Experiments.

The fishes have been weighed before each experiment and put in the experimental tray in their own aquarium water. The electrical activity of the electric organ has been monitored, recorded with the FM magnetic tape recorder, its amplitude measured on the calibrated oscilloscope, and a photo taken. After a few minutes, the water was discarded from the experimental tray and the anaesthetic was introduced with a funnel through one of the holes in the tray cover. The time of introducing the anaesthetic has been marked with the aid of a timer and also recorded on the magnetic tape and in our records. The effect of the anaesthetic on the fish, its behavior and respiration were constantly observed. The electric activity has been monitored and from time to time a photo has been taken from the oscilloscope. The moment in which the fish was anaesthetized completely has been recorded. The fish respiration and electric activity (amplitude, wave form and repetition rate) were constantly observed. If necessary, the anaesthetic has been immediately discarded and fresh aquarium water has been introduced in the experimental tray with adequate aeration for the fish. This moment has been recorded and the recovery time of the fish has been marked. The electric activity also has been monitored. When the fish was considered completely recovered, it was returned to its own aquarium. Sometimes because of the long time a fish was anaesthetized, it has been returned from the experimental tray in a net floating in its own aquarium with adequate aeration with bubble stones under the net.

Experiments were performed on five specimens of Sternarchus albifrons (No. 2, 3, 4, 6 and 7). In order to assess the effectiveness and dosage of the "MS 222 Finquel" tricaine methanesulfonate, we recorded the electrical activity before, during, and after anaesthesia. The frequency of the electric organ will drop immediately after adding the tricaine methanesulfonate to the water in a special tray, provided with stainless steel electrodes set at a distance of one cm from one another over the length of the lucite tray. The fish were restricted by a partition and a "U" shaped lucite device placed on top of the fish. Figure 14 shows the decrease in frequency

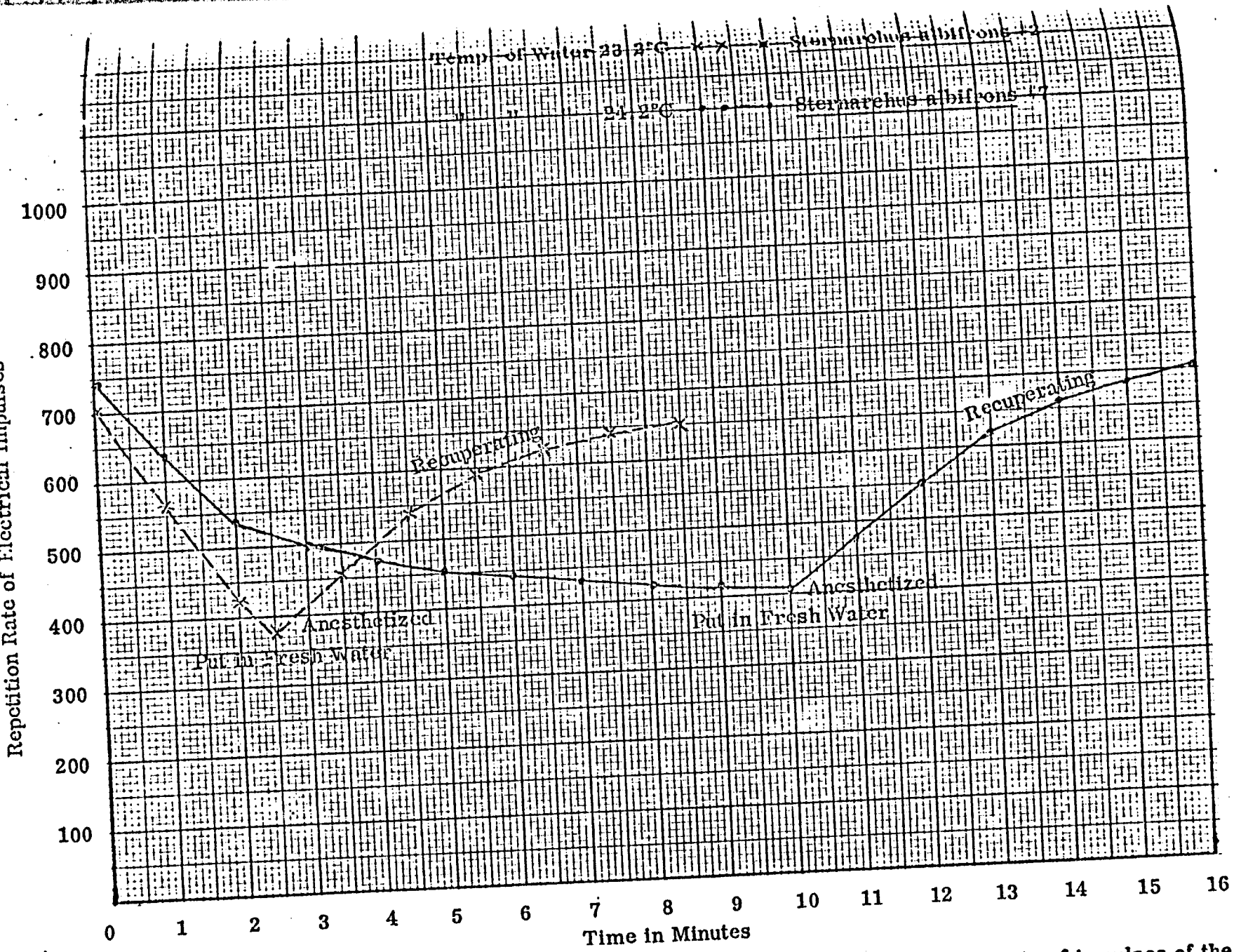


Figure 14. Effect of tricaine methane sulfonate solution 1:10000 in water on the repetition rate of impulses of the electric organ of Sternarchus albifrons to its complete anesthesia and recuperation after putting the fish in fresh water.

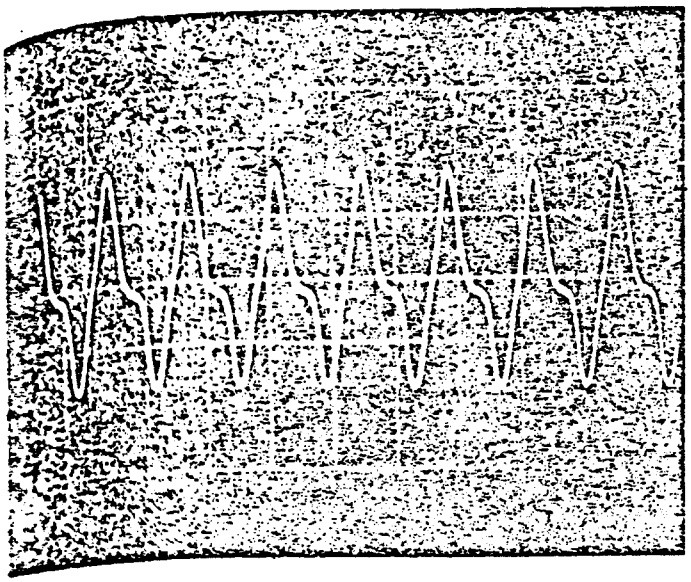
with time when the fish has been kept in a 1:10000 MS222 anaesthesia solution in water and the return of the impulse rate after the solution has been exchanged with fresh water. The electrical activity of the electric transmitting organ was recorded on a Hewlett-Packard four-channel FM magnetic tape recorder to be played back and analyzed later. Figure 15 shows the electric activity before anaesthesia; Figures 16, 17, 18 and 19 show the electric activity during anesthesia; and Figures 20 and 21 show activity during the recuperating process.

B. RESULTS AND DISCUSSION

From seven anaesthetic agents we tried, six were inadequate because the repetition rate of the electric signal was affected. Only the thiopental sodium Abbot (pentothal sodium) does not influence the electric activity of Sternarchus albifrons.

The thiopental sodium has been checked on five different fishes (same species). It has a very fast effect in a dosage of 1:10000 in water. The fish, after it has been anaesthetized, would remain as such for 2 to 4 hours lying very quietly. It recovers completely and the anaesthetic has no ill effect on the fish. We anaesthetized them repeatedly and after six months' time they are doing well. After the fish has been anaesthetized, it has to be put in aerated fresh water where it can remain for hours. Figures 23, 24, 25 and 26 show that the repetition rate of the electric signal has not been affected in over 40 minutes. The only change was produced by a slight decrease in water temperature (from 720 pulses to 700 pulses) of about 0.4 C. Sternarchus albifrons is very sensitive to changes in water temperature. It will increase the repetition rate of the electric signal for an increase in water temperature and it will decrease the repetition rate for a decrease in water temperature. For every degree Centigrade, it may change the repetition rate by about 50 to 80, depending on the particular fish.

Thiopental sodium or Sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate has the chemical formula $C_{11}H_{17}NaN_2O_2S$, a molecular weight of 264.33 and is a yellowish-



:5. Sternarchus albifrons #7 normal elec-
-al activity. Water temp. 24.2°C, time:
msec/cm, 5 mV/cm.

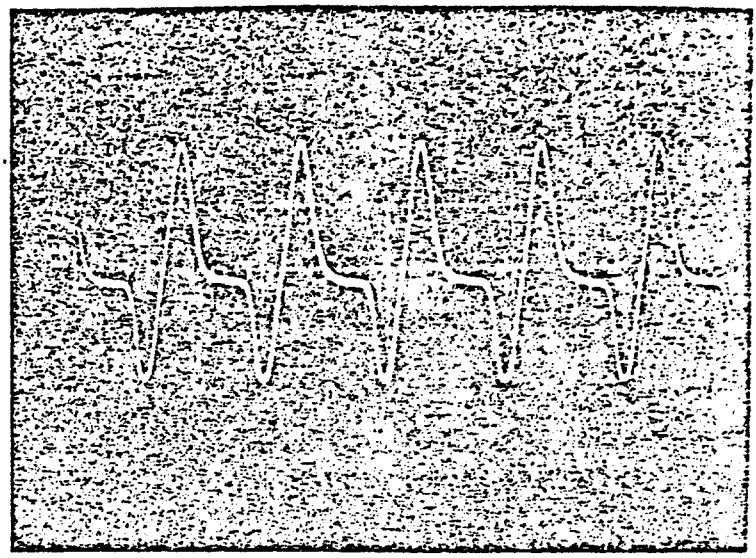
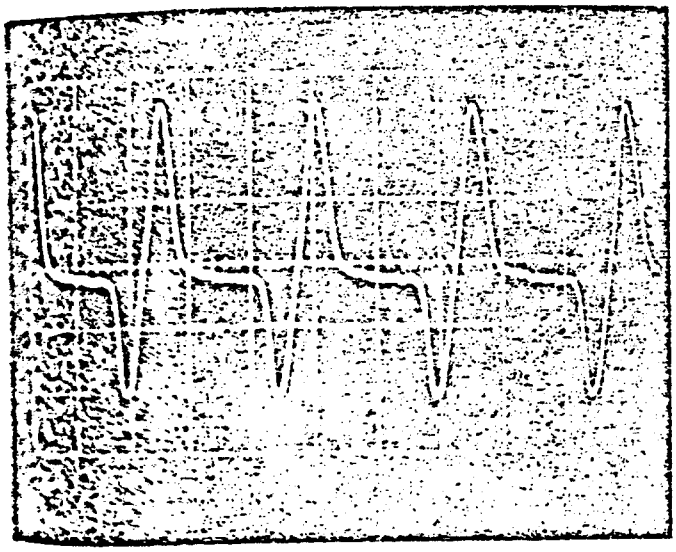


Fig.16. Sternarchus albifrons #7 in anes-
thetic tricaine methane sulfonate 1:10000.
Water temp. 24.2°C, time: 1 msec/cm,
5 mV/cm, 1 minute.



Sternarchus albifrons #7 in anes-
tricaine methane sulfonate 1:10000.
temp. 24.2°C, time: 1 msec/cm,
/cm, 2 minutes.

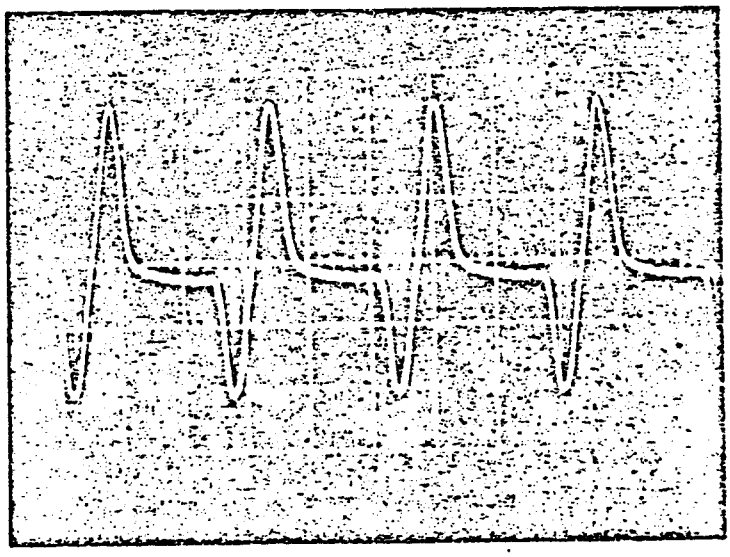
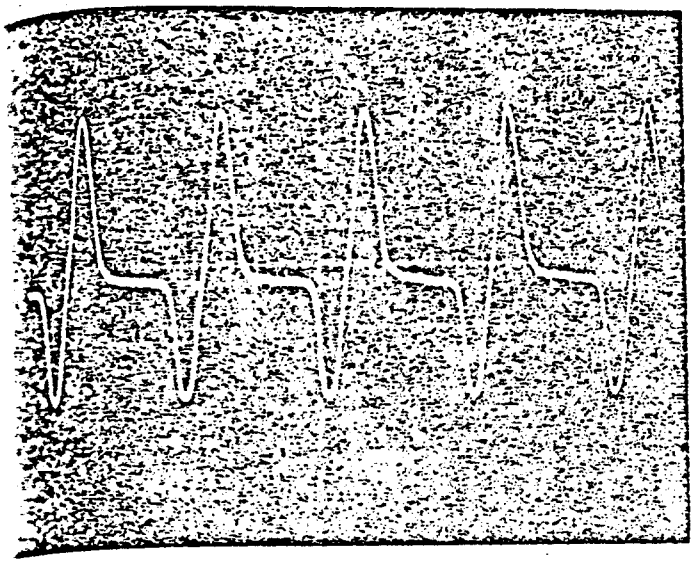


Fig.18. Sternarchus albifrons #7 in anes-
thetic tricaine methane sulfonate 1:10000.
Water temp. 24.2°C, time: 1 msec/cm,
5 mV/cm, 4 minutes.



:9. Sternarchus albifrons #7 in anes-
:ic tricaine methane sulfonate 1:10000.
:ter temp. 24.2°C, time: 1 msec/cm,
=V/cm, 5 minutes.

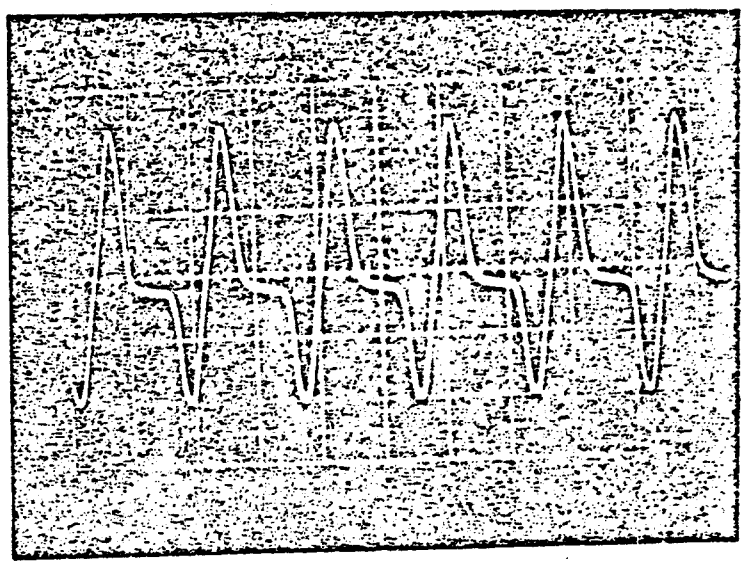
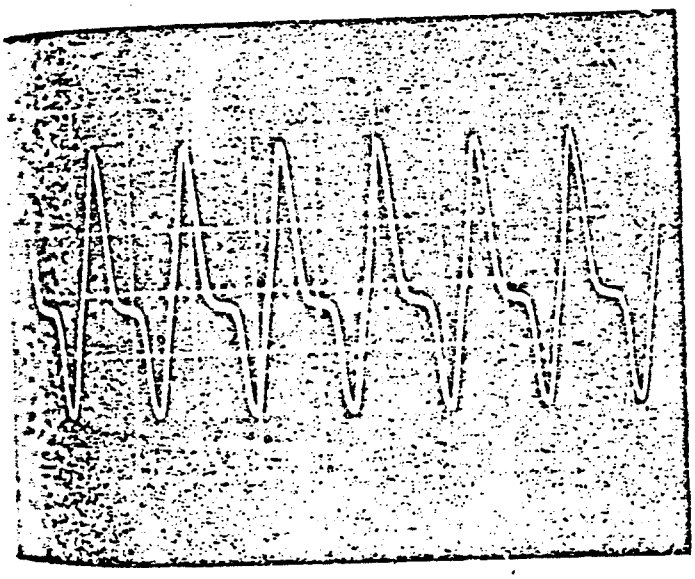


Fig. 20. Sternarchus albifrons recuperating in fresh water. Temp. 24.2°C, 5 minutes.



:. Sternarchus albifrons recuperating in
: water. Temp. 24.2°C, 8 minutes.

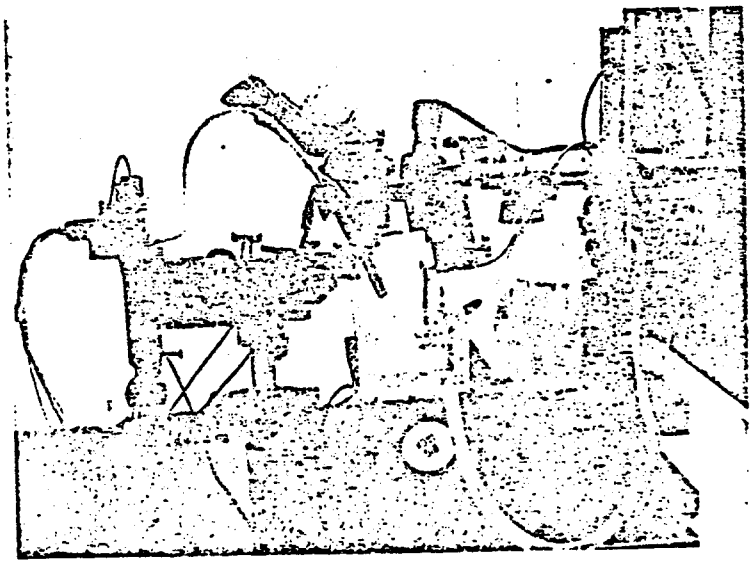


Fig. 22. New set-up for microelectrode recording.

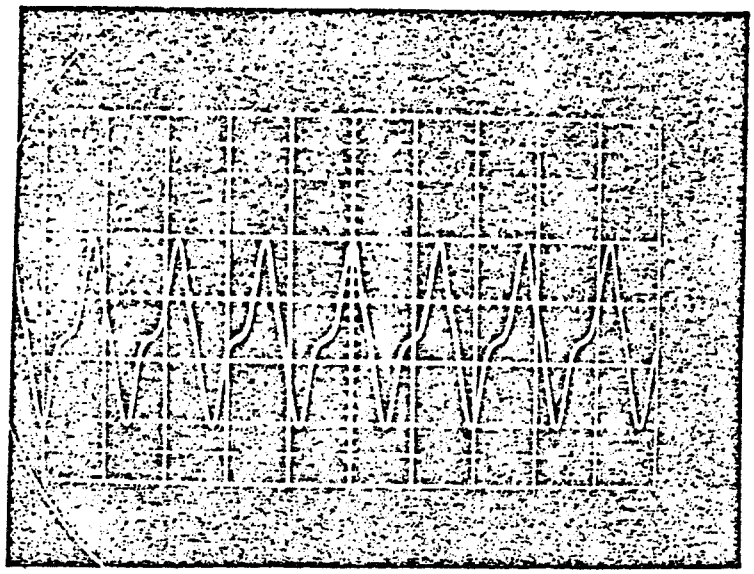


Figure 23. Sternarchus albifrons No. 1 just before anaesthesia with thiopental sodium. Electric signal rate: ≈710.
Sweep 1 ms/cm, Gain 10 mv/cm; Water Temp. 22.4°C.

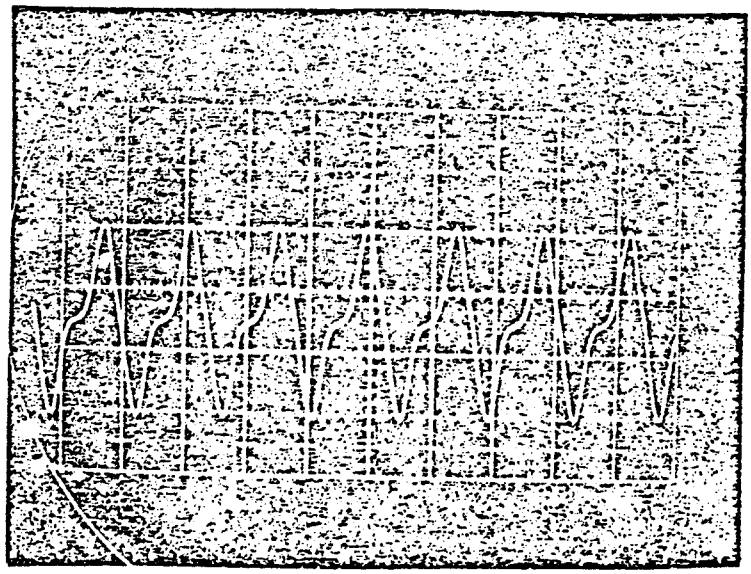


Figure 24. Sternarchus albifrons No. 1 anaesthetized with thiopental sodium 1:10000 (2 min.) 3.5 min. in fresh water but still completely immobile. Electric signal rate: ≈705.
Sweep 1 ms/cm, Gain 10 mv/cm; Water Temp. 22.35°C.

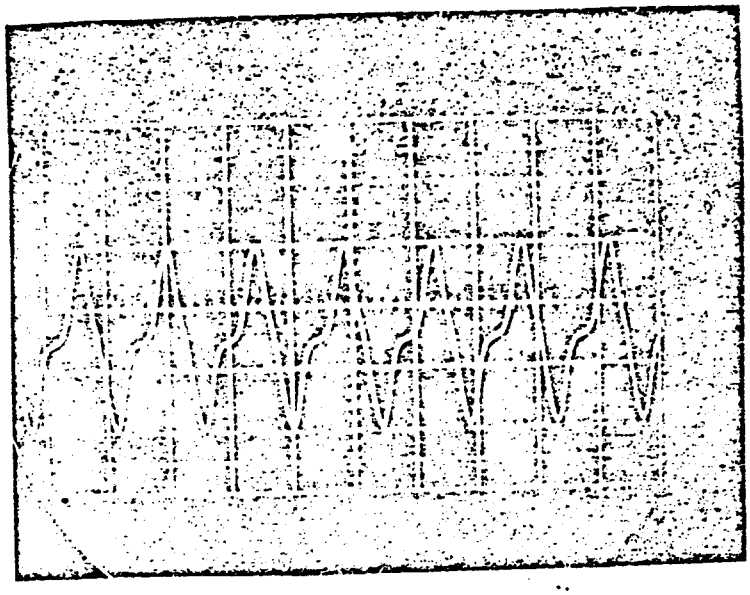


Figure 25. Sternarchus albifrons No. 1 anaesthetized with thiopental sodium 1:10000 (13.5 min.). Electric signal rate: ≈ 700. Sweep 1 ms/cm, Gain 10 mv/cm, Water Temp. 22.3°C.

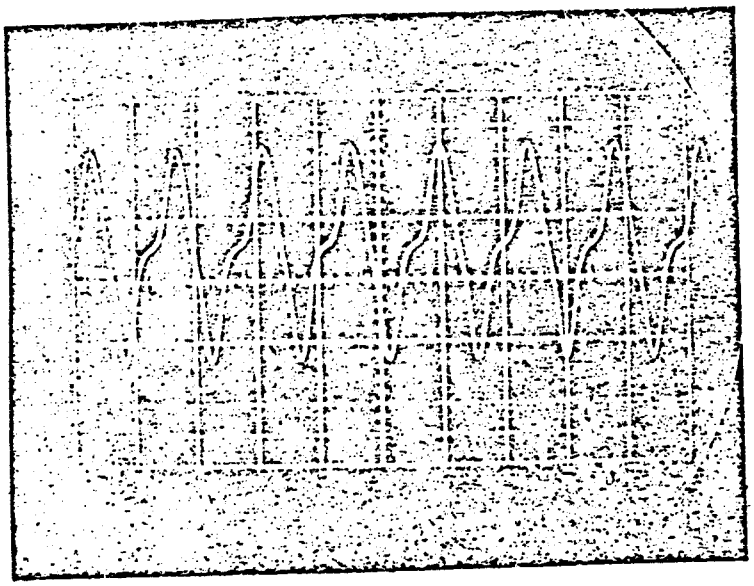


Figure 26. Sternarchus albifrons No. 1 anaesthetized with thiopental sodium 1:10000. 37.5 min. in fresh water. Electric signal rate: ≈ 700. Sweep 1 ms/cm, Gain 10 mv/cm, Water Temp: 22.3°C.

white, hygroscopic power, soluble in water and alcohol and is a strongly alkaline solution. The Abbot preparation is of nonhygroscopic crystals[46].

Tubocurarine chloride can be administered by intra-abdominal injection. There is no interference between tubocurarine and thiopental sodium.

The stability of thiopental solutions depends upon several factors, including the diluent and conditions of storage. It is recommended to keep them under refrigeration and tightly stoppered.

"Finquel" or "MS222" is a meta-amino-benzoic-acid-ethyl-ester in the form of tricaine methane-sulfonate and has the chemical formula $C_{10}H_{15}NO_5S$ with a molecular weight of 261.31 and is produced as fine needles, soluble in water. It is slightly acid and is stable to boiling.

Finquel 1:10000 in aquarium water would affect the repetition rate of Sternarchus albifrons and make it decrease in 10 minutes from 780 to 440 (Figures 27 and 28).

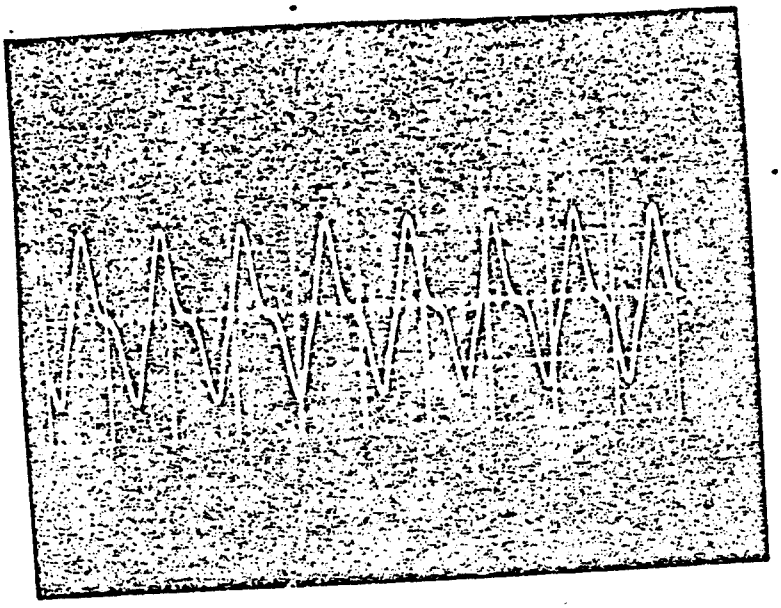


Figure 27. Sternarchus albifrons No. 2 in Aquarium Water.
Electric Signal Repetition Rate: ≈ 780.
Amplitude 25 mv, sweep 1 ms/cm, water temp. 22.4°C. 8/31/72.

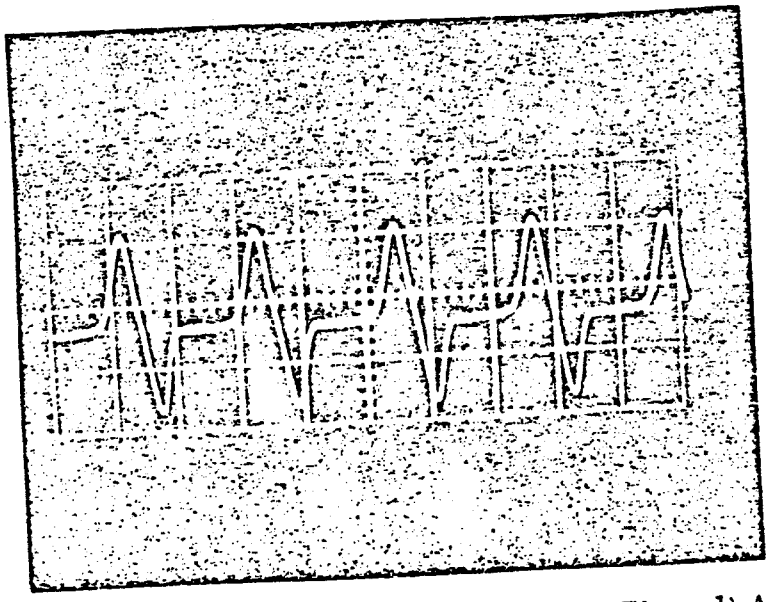


Figure 28. Sternarchus albifrons No. 2 in MS-222 (Finquel) Anaesthetic
Solution 1:10000 in Water. Electric Signal Repetition Rate
After 10 Minutes in the Anaesthetic: ≈ 440.
Amplitude 38 mv, sweep freq. 1 msec/cm, water temp. 22.4°C, 8/31/72.

IV. TECHNICAL DISCUSSION

Four specimens of Sternarchus albifrons have been used for establishing methods to record the autorhythmic activity of their electroreceptors (Figures 29, 30). The fishes have to be anaesthetized and curarized in order to avoid twitching of the muscles during microelectrode recordings. We tried the effects of d-tubocurarine on Sternarchus albifrons. Hagiwara, et al[42], recommended 0.05 to 0.1 mg curare/fish and Enger and Szabo[43] recommended 0.03 mg d-tubocurarine/g fresh fish weight. Both used (MS 222) tricaine methanesulfonate for anaesthesia (1:150,000). We found that the quantities given did not correspond in our case.

The weight of the Sternarchus specimens varied between 16 g and 19 g. One specimen (16 g) received 0.5 mg d-tubocurarine intra-abdominally. The fish was paralyzed in one minute. Electrical activity of the main electric organ subsided after 20 minutes and the fish was dead after another ten minutes. All the time the fish was kept in a 4000 ml beaker with medicated aquarium water at a pH of 7.0 and a temperature of 23.0°C. An aerator stone provided the necessary air.

A second specimen of Sternarchus (also 16 g) has been anaesthetized with MS 222, 1:25,000. One gram of MS 222 has been dissolved in 1000 ml distilled H_2O as stock solution. Then this was diluated to the proper amount in a 4000 ml beaker by adding aquarium water with merbromine and acriflavine added as disinfectants. The MS 222 dilution of 1:150,000 would not affect the fish in over one hour. The dilution of the MS 222 was reduced to 1:25,000. After 20 minutes in the MS 222 solution the fish was injected intra-abdominally with 0.05 mg of d-tubocurarine 1 mil solution. The fish was paralyzed in three minutes. For one hour it gave a good strong electric signal of the main electric organ, after this gradually it diminished in strength and in another hour the fish was dead.

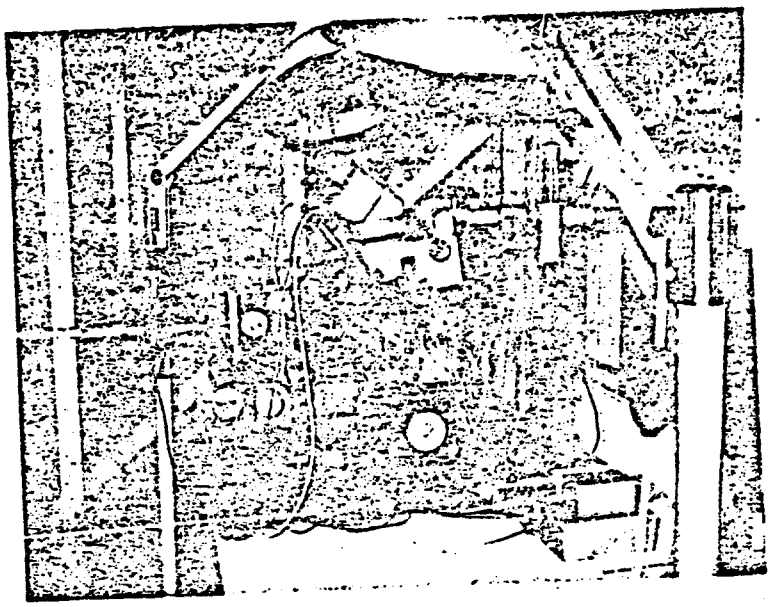


Figure 29. Set-up for recording the autorhythmic activity of electroreceptors.

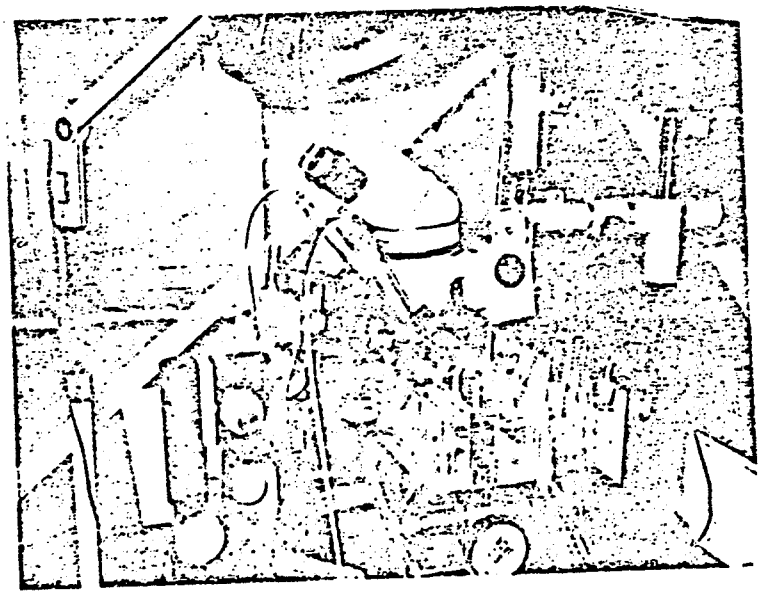


Figure 30. Close-up of the set-up for recording the autorhythmic activity of electroreceptors.

y the data given by the previous authors did not correspond for our

mens.

third specimen of Sternarchus (18 g) was first anaesthetized in MS 222
,500 for 20 minutes after this it was injected intra-abdominally with 0.1 ml
.05 mg/ml d-tubocurarine (Figure 31). The fish has been placed then in a
cial recording tray with circulating aerated water with merbromine, acri-
vine and MS 222, 1:75,000. Recording from the electroreceptors has been
tempted with a neutral electrode in the tray water and a silver-silver chloride
icroelectrode placed on a tonic, ampullary electroreceptor (Figure 32). The
trong electric signal from the main transmitting electric organ masked the weak
autorhythmic activity of the electroreceptors. This specimen has been returned to
its tank after 30 minutes and recovered almost instantly and is doing well since then.

Another fourth specimen of Sternarchus (19 g) received the same treatment as the
third specimen. This time instead of using the neutral electrode in the tray water, a
silver-silver chloride wire loop 1/4 in. diameter was placed around the tonic,
ampullary electroreceptor (Figure 33). This time the main electric organ signal
did not mask the recordings of the authorhythmic activity of the electroreceptor.
After 40 minutes, the fish was returned to its tank and recovered almost instantly,
like the previous specimen, and is doing fine without any ill effects since then (Figure
34).

The microelectrode recording experiments were performed in a shielded room from
which all a-c instruments, plugs and cords were eliminated and the shielded room
then constituted a floating ground. Illumination of the specimen was done by a micro-
scope lamp fixed on the stereo-microscope and connected to a 6 volt car battery. All
heaters were taken out of the water and all amplifiers were connected to batteries.
Other equipment, such as the microscope support, the microscope, the jack, the

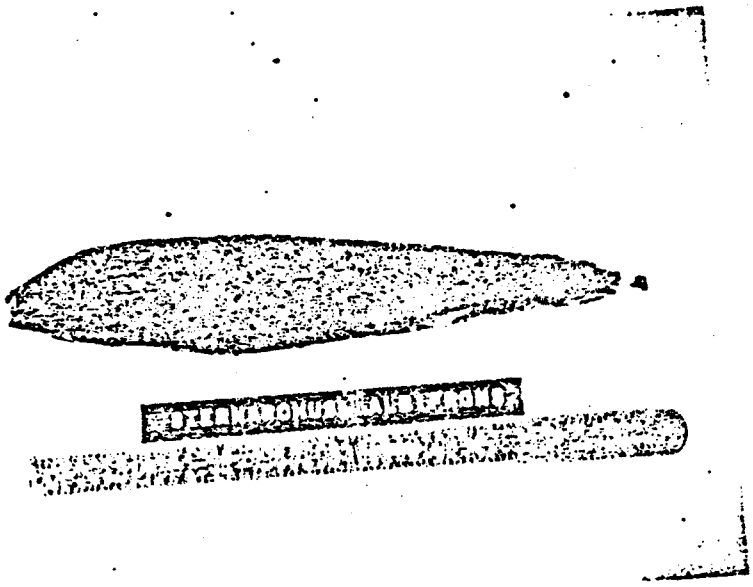


Figure 31. Sternarchus albifrons specimen after d-tubocurarine injection.

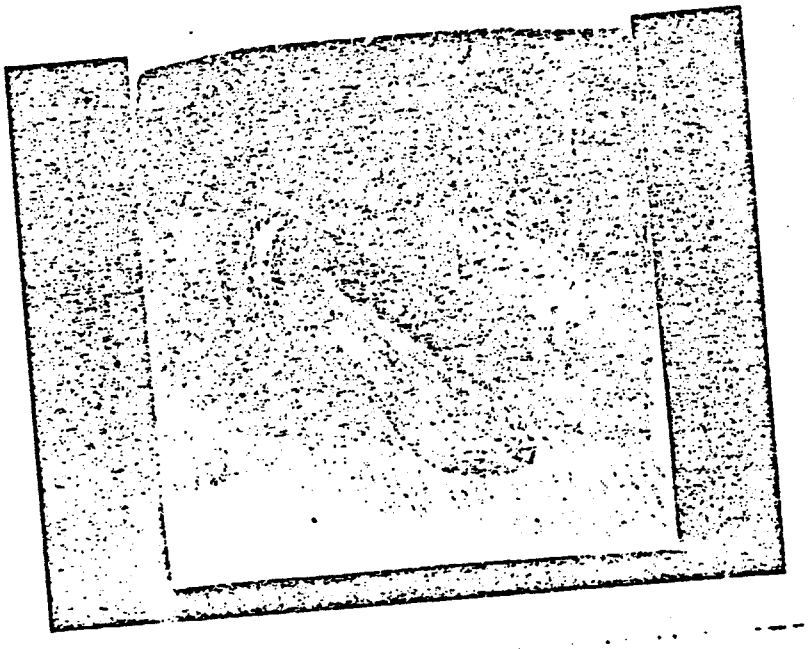


Figure 32. Ampullary, tonic electroreceptors of Sternarchus albifrons. The white points are ampullary receptors. The dark points are tuberous, phasic receptors.

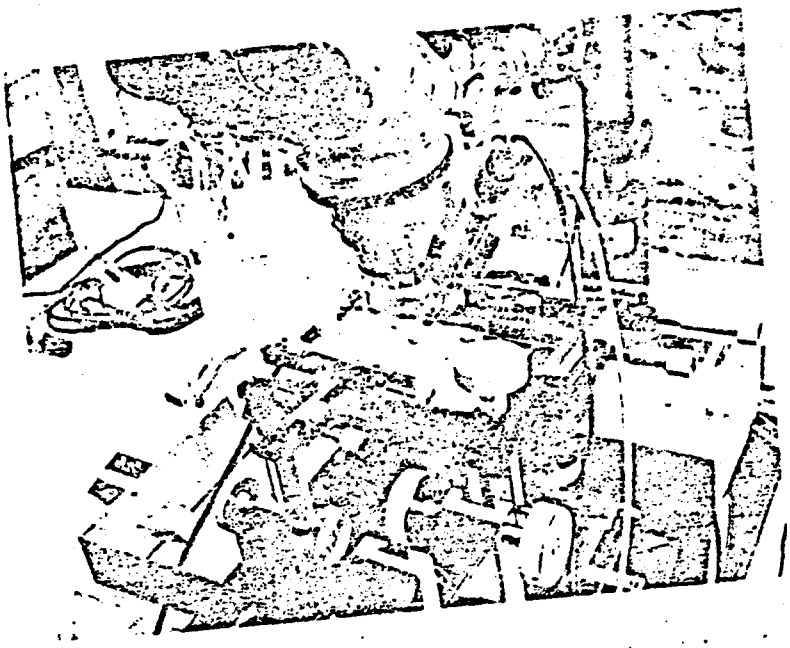


Figure 33. Set-up for recording autorhythmic activity of ampullary electroreceptors of Sternarchus albifrons. The silver-silver-chloride wire loop neutral electrode is visible.

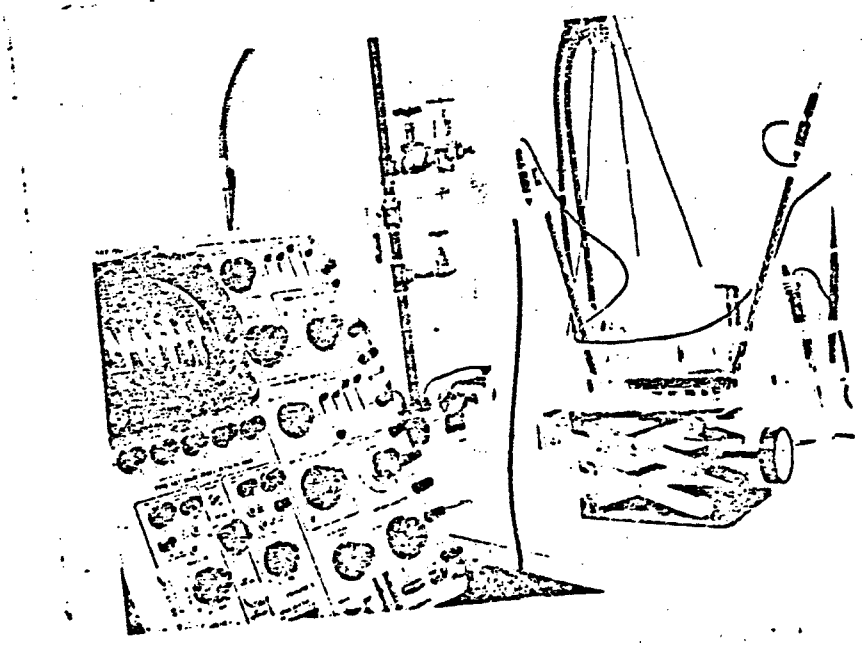
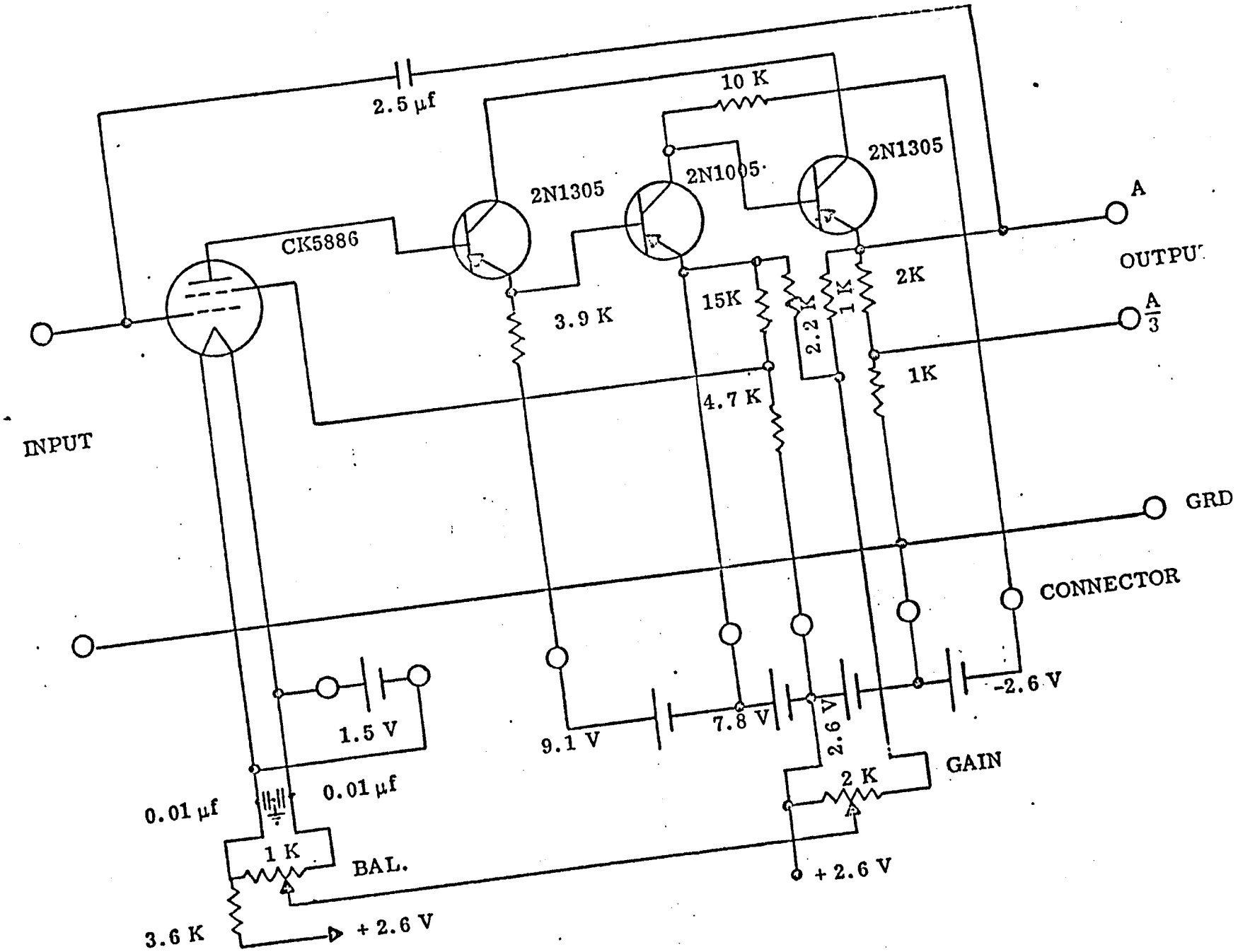


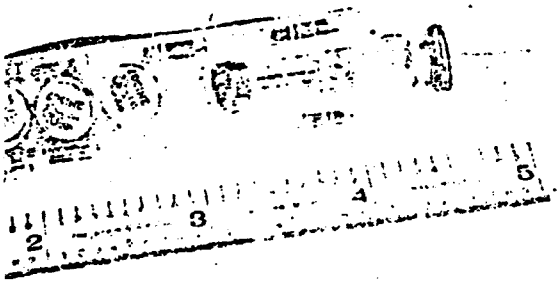
Figure 34. Anaesthetized, curarized Sternarchus albifrons in the recording tray. Carbon electrodes record the activity of the transmitting organ as seen on the oscilloscope.

plifiers, etc., was connected to one point ground and to the Tektronix T22 modified fferential amplifier ground. The microelectrode amplifier and support was changed om the integrated circuit model used before to an electrometer tube type and a three ansistor amplifier which is less sensitive to the change in impedance produced by light movements of the fish than the previous one. The microelectrode, a silver-silver-chloride, platinum, silver-chloride electrode of 0.5 micron tip diameter, was positioned on the electric receptor inside the grounding loop. Figure 22 shows the recording setup and Figure 35 the schematic of the microelectrode amplifier.

At the same time, the electric activity of the receptors was displayed on one of the traces of a dual scope. The activity of the electric transmitting organ was recorded with two carbon electrodes placed at the end of the tray and connected to another Tektronix T22 amplifier and displayed as a second trace on the scope. A Hewlett-Packard four channel FM tape recorder was used to record the electrical activity of the electric receptors on a magnetic tape. It can be played back and analyzed at a later time. Photos were made during the recording. Figure 37 shows the activity of a synchronous tonic electroreceptor (in a previous report we mentioned that we recorded from a nonsynchronous tonic electroreceptor), and Figure 38 shows the electric activity of a nonsynchronous phasic electroreceptor. After finishing the experiment, the fish was injected with neostigmine methylsulfate $1:10^6$ to counteract the effect of the D-tubocurarine. The fishes recuperated in a few minutes and are doing well.

Another experiment was performed with two Gymnarchus niloticus, Cuv. Each of these fishes was placed in 25 gallon water tanks with lucite trays raised to approximately 4 inches from the top. Gymnarchus niloticus is an air breather and if left in a deep tank must expend excessive energy to swim to the surface for breathing. The tank is kept clean by lucite plates provided with holes to let dirt fall to the bottom where it can be vacuum-cleaned very easily through special holes. The tanks of


2.5 µf
10 K
2N1305
2N1005
2N1305
A
OUTPUT
CK5886
3.9 K
15K
2.2K
1K
2K
A/3
1K
4.7 K
INPUT
GRD
CONNECTOR
1.5 V
9.1 V
7.8 V
2.6 V
-2.6 V
0.01 µf
0.01 µf
2 K
GAIN
1 K
BAL.
+2.6 V
3.6 K
+2.6 V



High impedance microelectrode cathode follower preamplifier.

3. High impedance microelectrode
de follower preamplifier.

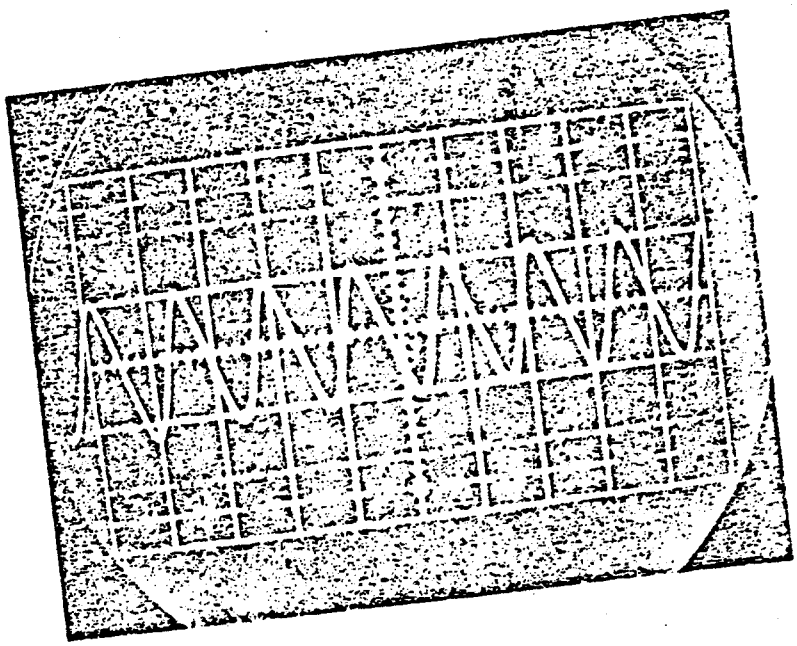
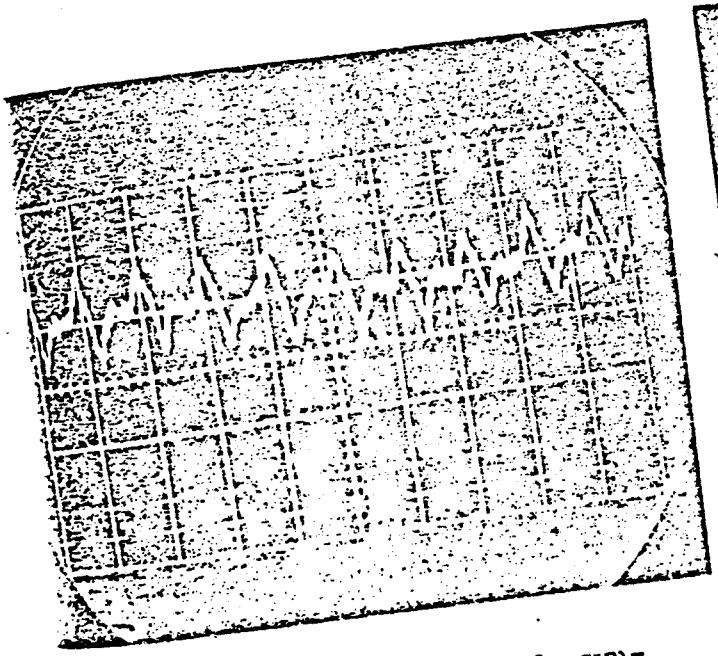


Fig. 37. Sternarchus albifrons #6. Synchronous tonic electroreceptor. Microelectrode recording, 1 msec/cm, 100 mV/cm.



38. Sternarchus albifrons #7. Nonsynonous phasic electroreceptor. Microtrode recording, 2 msec/cm, 100 mV/cm.

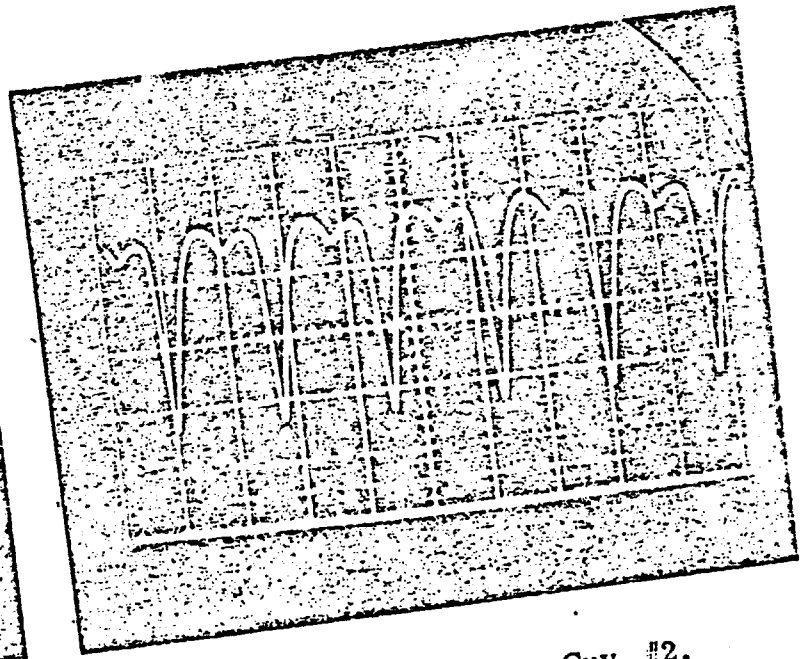


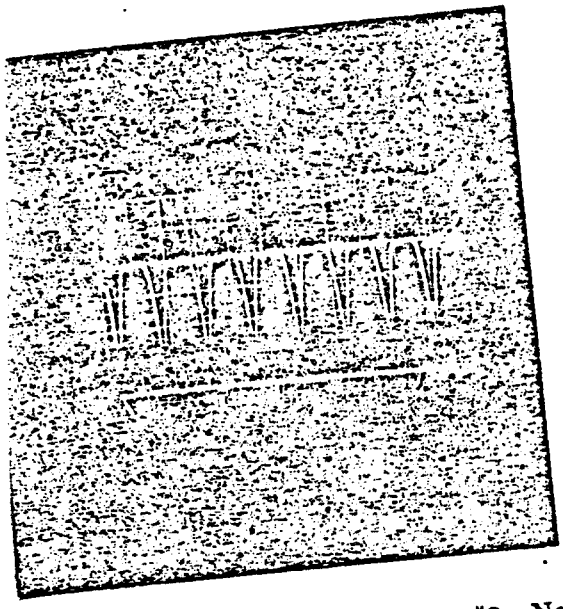
Fig. 39. Gymnarchus niloticus Cuv. #2. Normal electric activity of the transmitting electric organ. Not connected with the tank of another Gymnarchus. 2 msec/cm; 1 mV/cm.

nnarchus niloticus are approximately three feet apart. . The covers and outside .magnetic stainless steel frames were all grounded and so was the tank through ·bon electrodes placed in the filters.

/o carbon electrodes in lucite tubes (with holes) were placed at a distance of 15 cm)m each other alongside the fishes. A third electrode was placed either midway :tween the recording electrodes or on one of the ends of these electrodes. The first :o electrodes for each fish were connected with the T22 Tektronix amplifiers and)nnected to two different Tektronix scopes. The third electrodes were connected) a DPDT switch which could actuate also a battery to raise the lower trace of the cope when the two electrodes in the two tanks were connected and hence a communica- ion link was established between the two Gymnarchus to study the effect of social nteraction and communication between them.

n Figure 39, the normal electrical activity of the electric organs of Gymnarchus lo. 2 and in Figure 40 the electrical activity of Gymnarchus No. 3 can be seen. 'igures 41 and 42 show that Gymnarchus No. 2 almost stopped the electrical activity)r ten to twenty seconds. Figures 43 and 44 show the modified activity of the im- ılses from Gymnarchus No. 3, and Figure 45 shows the modified activity of Gymnarchus). 2. When a metal rod was introduced in the tank of Gymnarchus No. 3, Gymnarchus). 2 would nervously move back and forth and eventually attack the electrode connected th the other fish. This is only the beginning of studying the communication between o Gymnarchus niloticus.

e Gymnarchus niloticus baby was sectioned and fixed in buffered formaldehyde (10%) about one month. Then it was decalcified with Kristensen's decalcifying solutions 24 hours. After this, it was dehydrated using ethyl alcohol, toluene, toluene with 'affin, and finally embedded in degassed paraffin with 56°C melting point.



0. Gymnarchus niloticus Cuv. #3. Normal
tric activity of the transmitting electric
an. Not connected with the tank of another
nnarchus. 2 msec/cm; 1 mV cm.

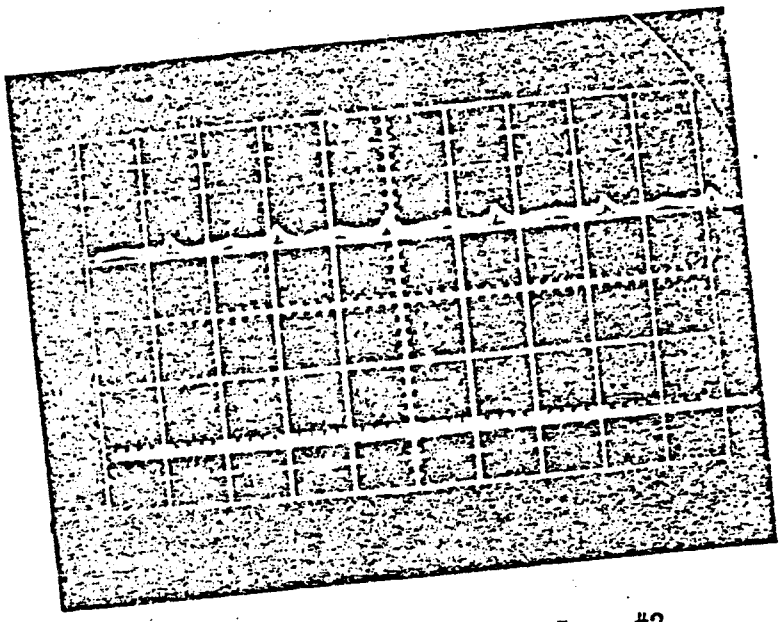
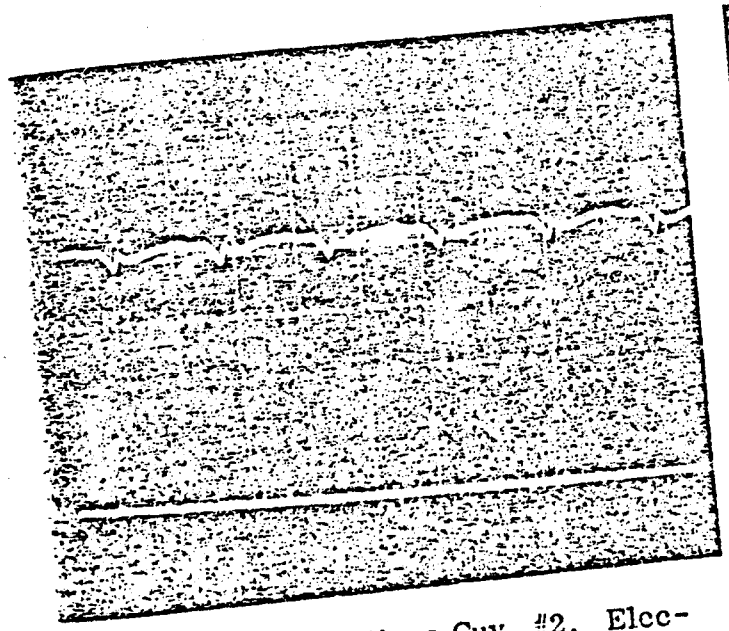


Fig. 41. Gymnarchus niloticus Cuv. #2.
Electric activity of the electric organ when
tank has been electrically connected through
carbon electrodes and a wire and switch with
the tank of another Gymnarchus. 2 msec/cm;
1 mV/cm.



2. Gymnarchus niloticus Cuv. #2. Elec-
activity of the electric organ when the
has been electrically connected through
bon electrodes and a wire and switch with
tank of another Gymnarchus. 2 msec/cm;
V/cm.

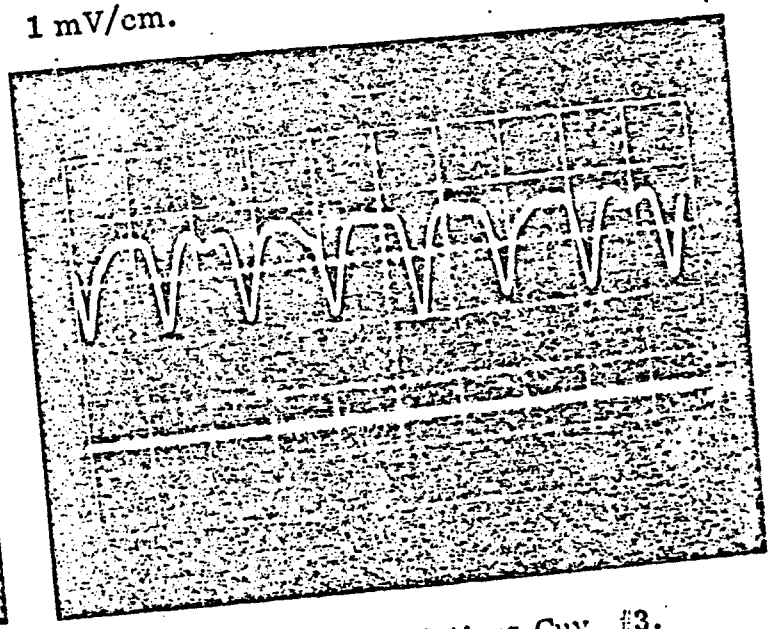
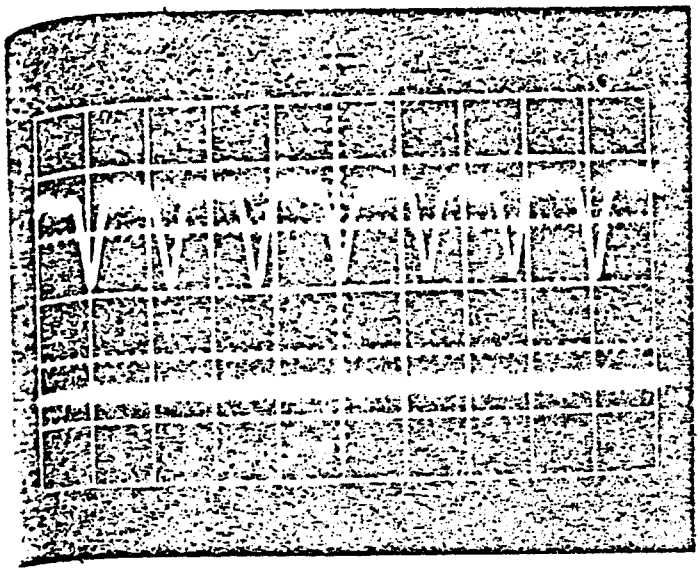


Fig. 43. Gymnarchus niloticus Cuv. #3.
Electric activity of the electric organ when
the tank has been electrically connected through
carbon electrodes and a wire and switch with
the tank of another Gymnarchus. 2 msec/cm;
1 mV/cm.



.44. Gymnarchus niloticus Cuv. #3.
lectric activity of the electric organ when
e tank has been electrically connected
rough carbon electrodes and a wire and
itch with the tank of another Gymnarchus.
msec/cm; 1 mV/cm.

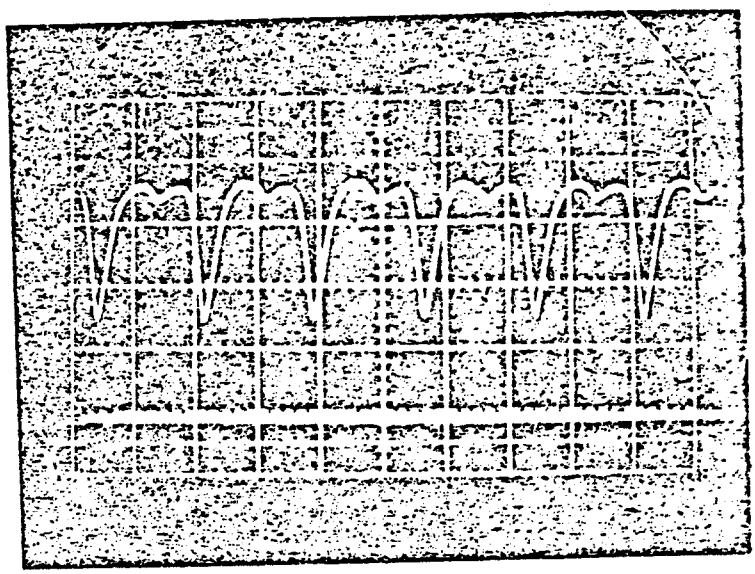


Fig. 45. Gymnarchus niloticus Cuv. #3. Electric activity of the electric organ when the tank has been electrically connected through carbon electrodes and a wire and switch with the tank of another Gymnarchus. 2 msec/cm; 1 mV/cm. After one minute, the fish switched to its normal activity but with a smaller amplitude.

An A. O. Spencer microtome has been used to cut 10 micron slices. They were stained with Hematoxylin-Eosin and mounted with Permount on microslides. There are about 27 microslides with four to five serially cut transversal slices numbered from the head in the direction of the tail: 1-1, 1-2, 2-1, 2-2, ..., 7-3, 7-4. A few photomicrographs are shown as Figures 46, 47, 48, and 49. The interpretation of the histological preparation will be made at a later date. (Figure 50)

Malapterurus electricus, the electric catfish (Figure 51) is different from the other lectric fishes because it does not obey Pacini's law. All the other electric fishes bey Pacini's law, according to which the innervated faces of the electroplates be-ome negative during the discharge, whatever the anatomical orientation of the organ.[47] his fact is due to the unique anatomy of Malapterurus. The electric organ of alapterurus forms a sort of loose jacket around the fish, instead of being embedded

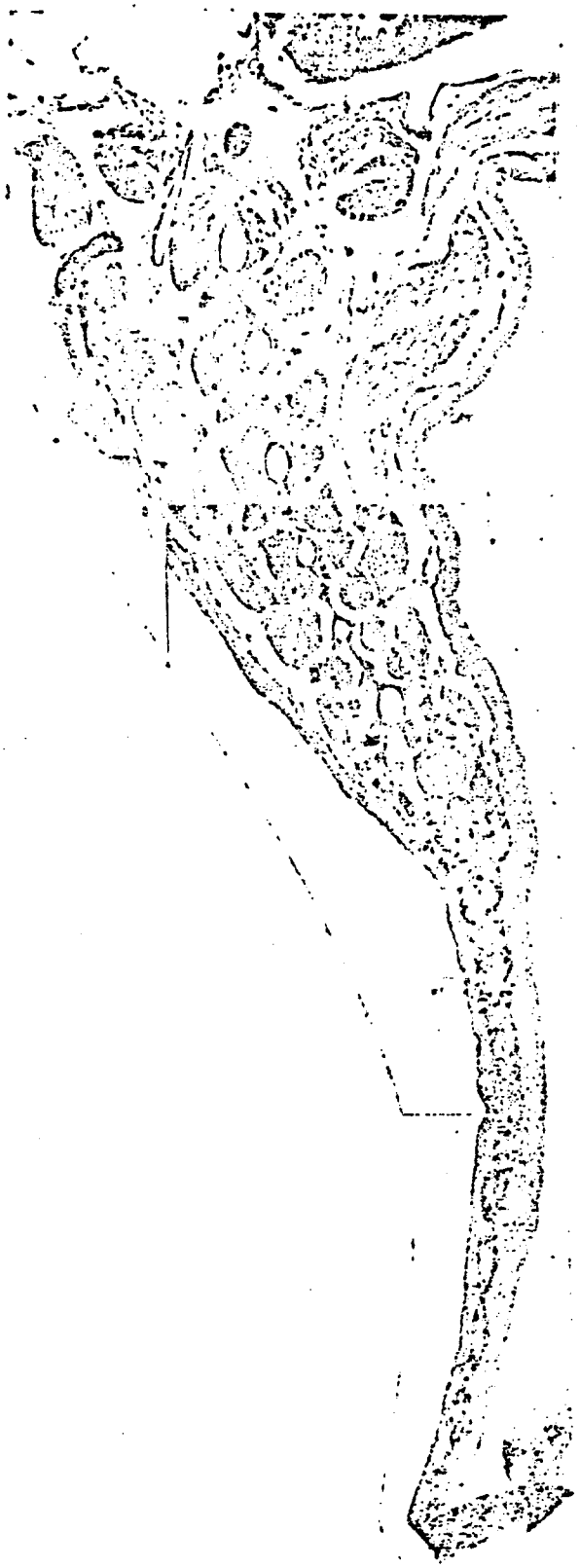
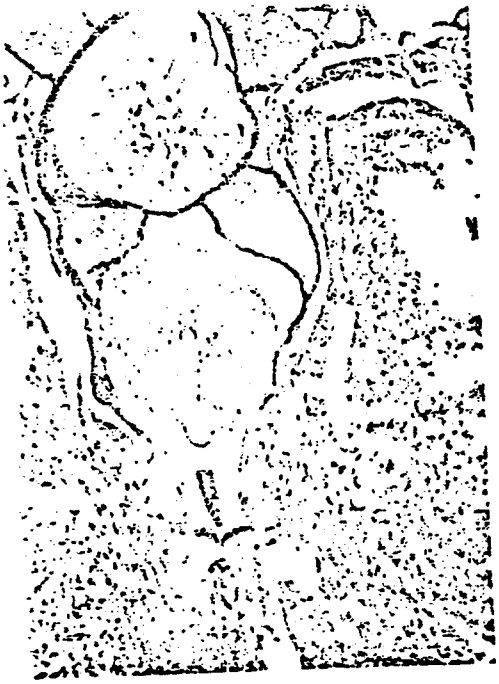


Figure 46. Gymnarchus niloticus, Cuv. (baby)
Transversal cut through the last 1/4 in the direction of the tail.
Fixation: 10% buffered formol



nnarchus niloticus Cuv., baby
s). Air bladder and spinal cord.
graph Nikon Phase-Interference
× 80. Fixed: Formaldehyde,
cifying. Hematoxylin-Eosin stain.

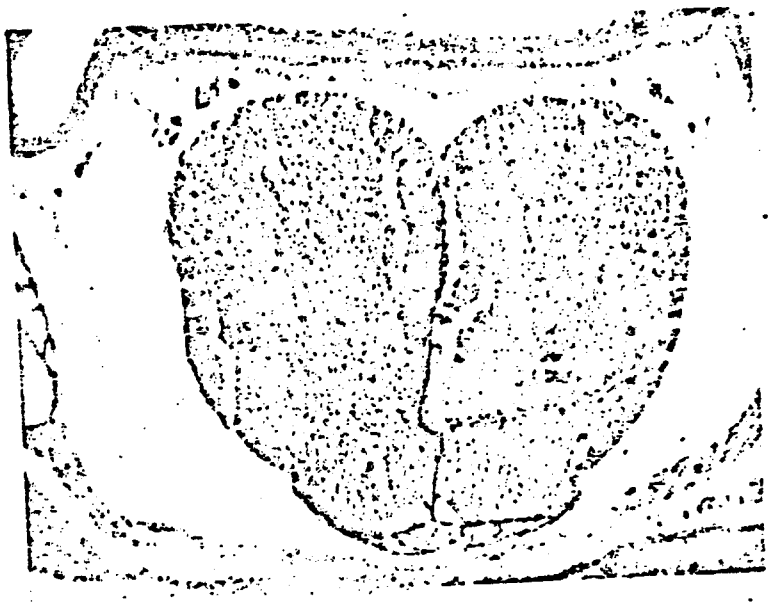
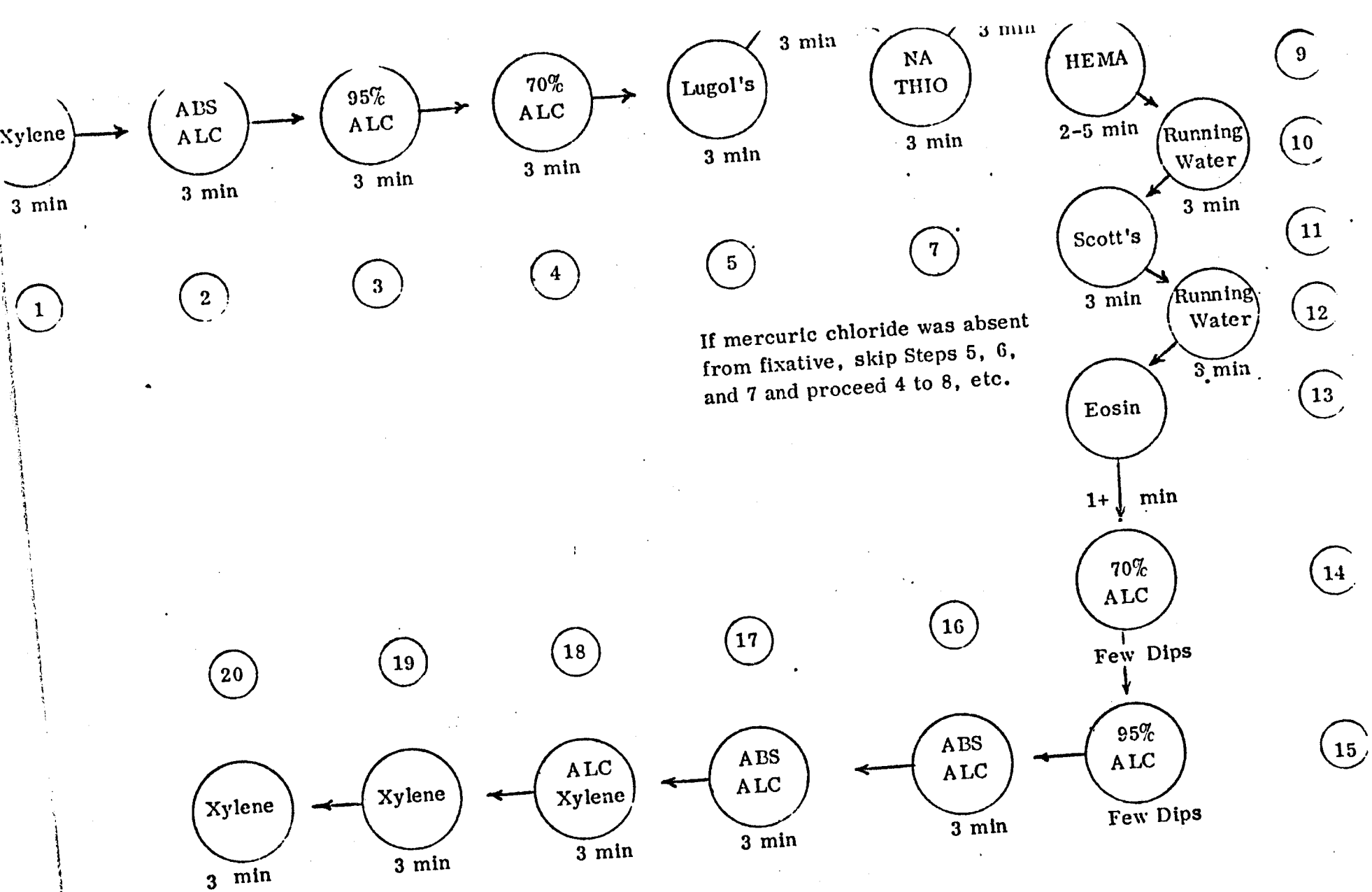


Fig. 48. Gymnarchus niloticus Cuv., baby (≈ 2 months). Spinal cord. Photomicrograph Nikon Phase-Interference Microscope × 200. Fixed: Formaldehyde, 10%. Decalcifying. Hematoxylin-Eosin stain.

narchus niloticus Cuv., baby
. Brain. Photomicrograph
-Interference Microscope ×
Formaldehyde, 10%. De-
Hematoxlin-Eosin stain.



If mercuric chloride was absent from fixative, skip Steps 5, 6, and 7 and proceed 4 to 8, etc.

Fig.50. DELAFIELD — HARRIS HEMATOXYLIN

thin the body muscle as in all the other electric fishes. The electric organ, depend-
; on the fish's size, forms a sheath from 2 to 10 mm or even thicker, in which the
ctroplates are stacked in the longitudinal direction arranged in a somehow non-
derly fashion, contrary to the other electric fishes. The electroplates are disks
about 1 mm diameter and 20 to 40 microns thickness and derived from myeloblasts.
the center of the caudal face of each electroplate, a thin stalk arises from a complex
agination. The end of this stalk makes contact with the branches of a single myelinated
ve fiber arising from a large nerve cell situated in the gray matter of the spinal cord
ween the levels of the first and second spinal nerve roots. As it can be seen on
ure 51, the nerve trunk which is heavily myelinated is easily identified. The way
which the electric organ is innervated plays an important role in the synchronization
chanism of the discharge.

Malapteruridae discharge consists of a long train of 15 to 30 monophasic pulses
to 2 msec duration in a total time-interval of approximately 100 milliseconds.

x to seven inch electric catfish could discharge impulses of approximately 200 V.
ny surprise, Dr. Earl Harold from the California Academy of Sciences and Stein-
lt Aquarium in San Francisco, told me that the two very large (approximately 2 to
ot) electric catfishes which the aquarium had did not discharge more than 100 volt
es.

surprising that the Malapteruridae we checked could put about 0.5 w/hr of energy
ach gram of electric tissue of the electric organ. It is our intention to investigate
fact more closely. The electric discharge of three strong fresh water electric
s Malapterurus electricus weighing between 55-65 g was measured when in the
r and out of water in the special tray with contacts. They discharged between
0 volts in water and between 150-190 volts out of water. The discharge consists
rsts of 5 to 7 impulses of 1.5 to 2 msec duration (Figures 52, 53, 54).

Figure 51 Malapterurus electricus. The Nile Electric Catfish.
(Shown at 1.5 Scale)

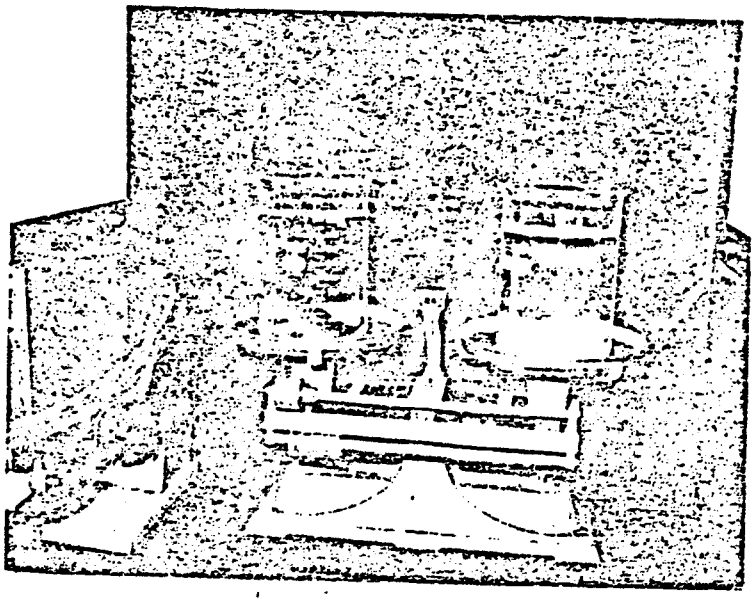


Figure 52. Malapterurus electricus, African strong fresh water electric fish on the scale: weight 61.5 g.

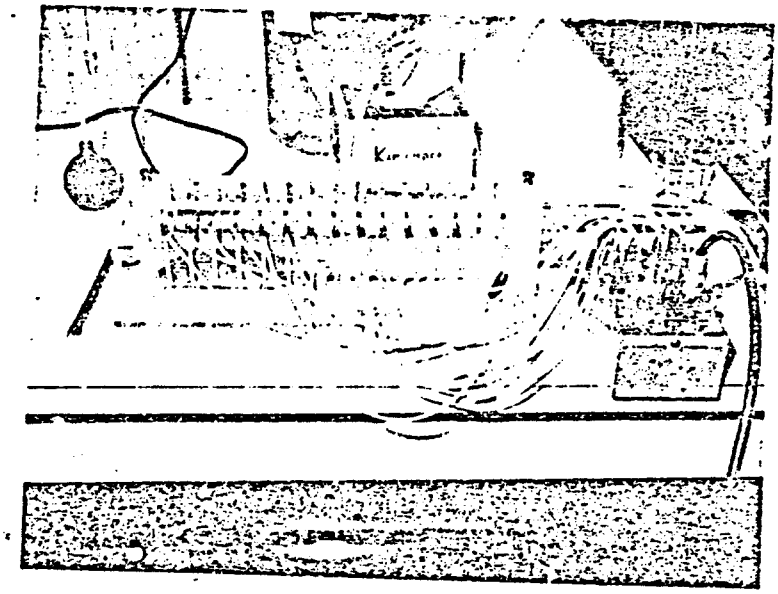


Figure 53. Device for measuring the voltage of the discharges of Malapterurus electricus.

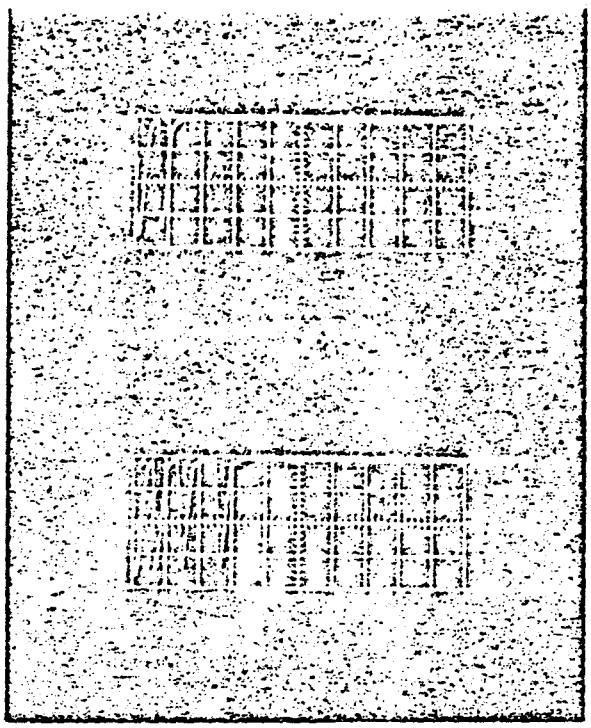


Figure 54. Discharges of the electric organ of Malapterurus electricus we have now in the laboratory. Vertical: 1 graduation = 50 V. Horizontal: 1 graduation = 5 msec.

In our synoptic Table No. 1, the past achievements and results of the study are mentioned, the proposed continuation work is shown, and the future objectives are delineated.

The multimodulation-multicoding communication system of electric fishes served as a nodel in the development of a communication technique to resist jamming.

'he physical analogs of a phasic and a tonic electroreceptor have been established for 'e Sternarchus albifrons, a South American fresh water weak electric fish of the high 'equency type (Figures 55, 56). The electroreceptors of other species have to be 'vestigated and the relationships of the various electroreceptors to the underwater 'ttern recognition system will be established.

: mentioned the effect of moving objects past the electroreceptors in one or another 'ection. Some of them would respond in one way for the forward direct (head to tail) increasing the rate of the impulses and in another way for the backward direction

TABLE 1

PAST, ACTUAL AND FUTURE STUDY PROGRAM OF UNDERWATER PATTERN RECOGNITION SYSTEM

SENSOR STUDY RESULTS

- Multi-modulation

PRELIMINARY ANALOG MODELS

- Asynchronous Phasic
- Asynchronous Tonic
- Synchronous Tonic

PHASE I

- Investigate electroreceptors of other species
- Establish relationship of various electric receptors
- Determine how receptors are used in navigation

PHASE II

- Design & conduct stimulus-response experiments in large tank
- Determine effective range
- Signal processing in noisy environment
- Improved physical analogs

STUDY OUTPUT

UNDERWATER PATTE RECOGNITION SIMULATION PLAN

OTHER SENSORS

- Mechanical
- Sonic
- Optic

PATTERN RECOGNI MODEL

- Cross Correlation
- Majority Decision

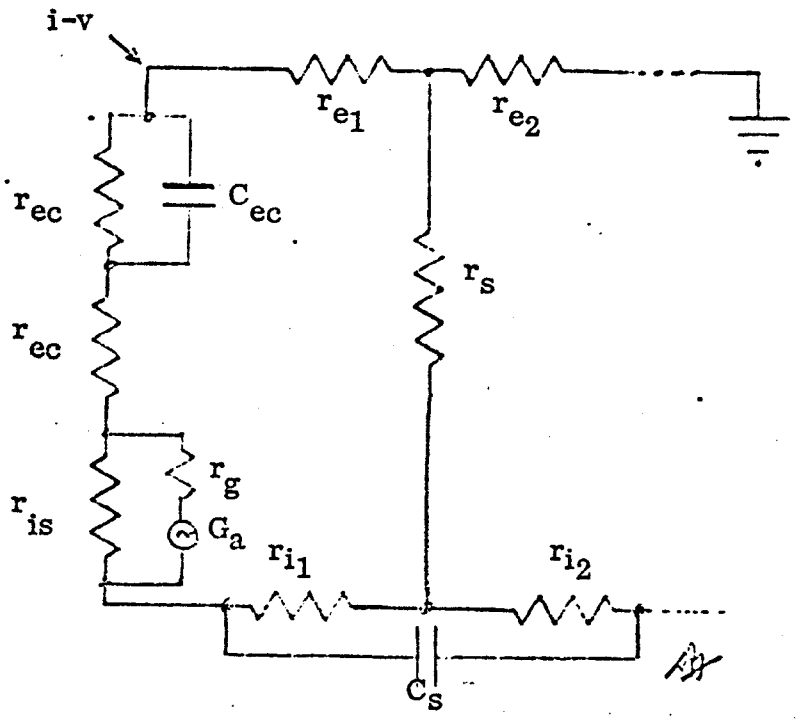


Figure 55. Tonic, ampullary electroreceptor. Physical analog.

	current and voltage	r_g	generator internal resistance
2	external resistance	G_a	autorhythmic generator
	external canal resistance	r_{i_1}; r_{i_2}	internal resistance
	external canal capacitance	C_s	internal receptor capacitance
	receptor inner face resistance	r_s	skin resistance

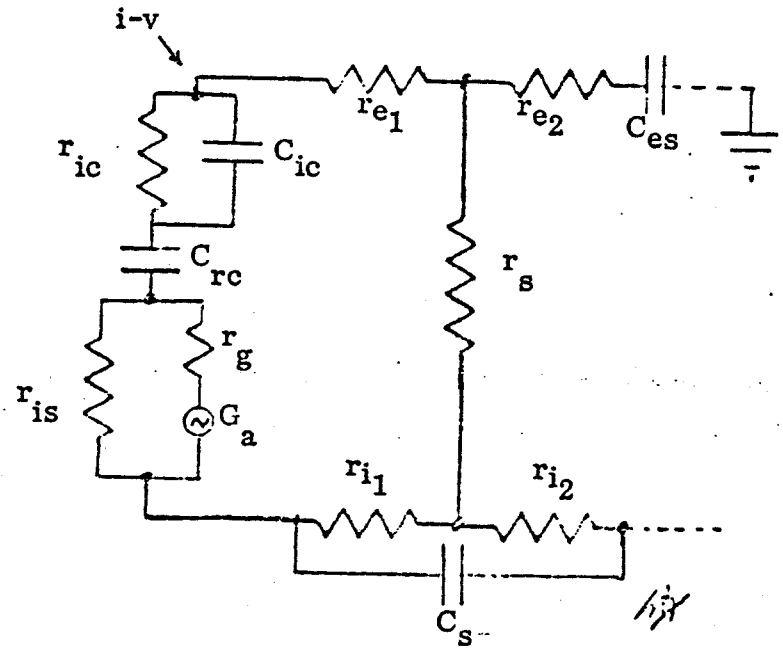
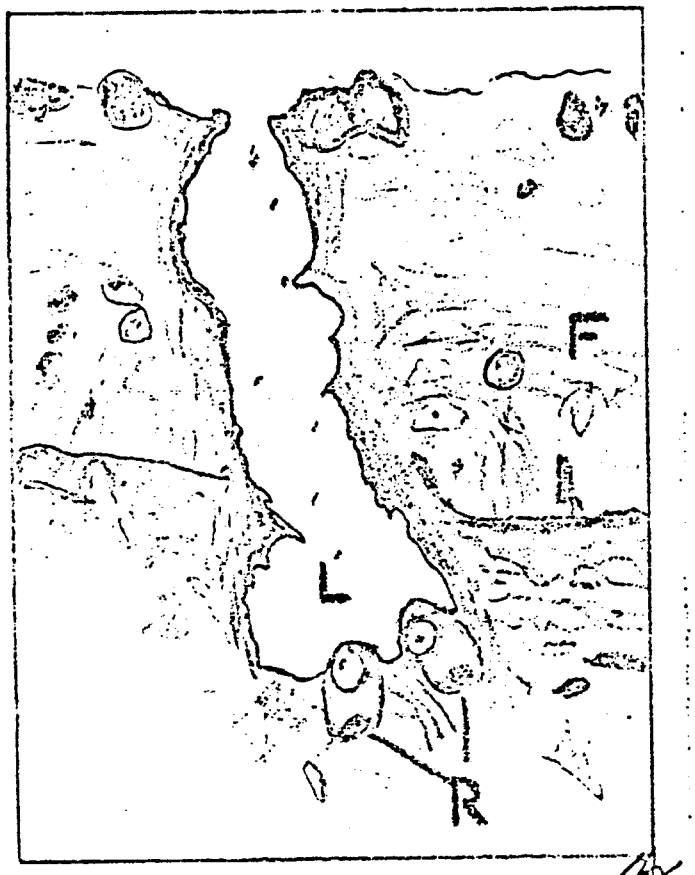


Figure 56 Phasic, tuberous electroreceptor. Physical analog.

current and voltage	r_g	generator internal resistance
external resistance	G_a	autorhythmic generator
internal canal resistance	r_{i_1}; r_{i_2}	internal resistance
internal canal capacitance	C_s	internal receptor capacitance
receptor cell capacitance	r_s	skin resistance
receptor inner face resistance	C_{es}	external skin capacitance

(tail to head) by decreasing the rate of impulses. Other electroreceptors will react to higher or lower conductivity than the medium (aquarium water) in a similar way. Obviously, the response of the electroreceptors in other electric fish species have to be assessed in order to get a better overall picture of the underwater pattern recognition process. Figures 57 through 63 show two different electroreceptors of electric fish. The results obtained from the electric receptors study will lead to a simulation plan of an underwater pattern recognition system. A passive hybrid underwater pattern recognition system block diagram is shown in Figure 64. In this block diagram beside the electric sensory receptors, the other types of lateralis line sensory receptors are combined to obtain a better cross-correlation of the different signals. Eventually passive optical sensory receptors will be added (for detecting, for example, changes in the bioluminescent organisms in the water when disturbed by a stimulus like heat, water turbulence, etc.).

With regard to the other lateralis line organs, Sternarchus albifrons, like other teleosts, have canal organs as well as superficial organs, called free neuromasts. Some particularities of these organs should be noted. Not all canal organs are in direct relation with the external environment. The sense organs do not always alternate with the pores of the canal[48]. In some cases, three sense organs are located between two pores. All three cupulae lie in a "watery" fluid filling the canal. The fluid seems to be a form of polymucosucharides. The canal is not free from one pore to another, but is obstructed between the front and second, and the second and third cupula-sense organ units by two epithelial plugs. Thus, the middle unit is not in direct continuity with the external environment (Ref. 23). It should be mentioned that Gymnarchus as well as Notopterus have a completely closed canal system. The free neuromasts found in the head of Sternarchus are relatively large, are protected by paired prominent epidermal flaps and have a histological structure similar to "big pit organs". As in the pit organs of Gymnarchus (Ref. 25), these free organs of Sternarchus are innervated by a bundle



L= LUMEN R= RECEPTOR CELLS

F= FLATTENED CELLS (After Szamier & Wachtel)

..re57. Tonic Electroreceptor South American Fresh Water Weak Electric Fish.

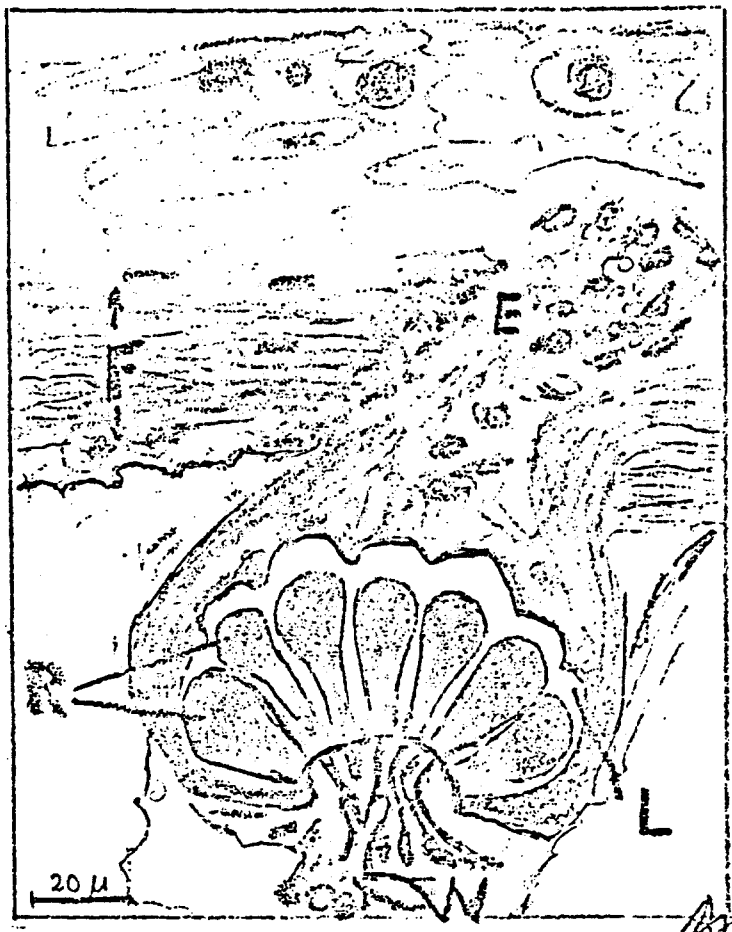
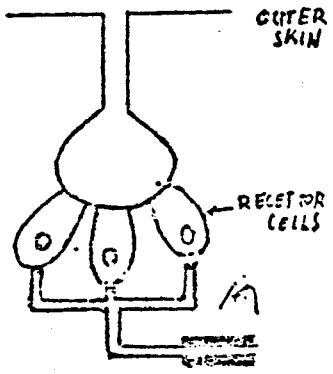


Figure 58. Phasic Electroreceptor South American Fresh Water Weak Electric Fish.



59. Ampullary tonic electroreceptor. (Schematic)

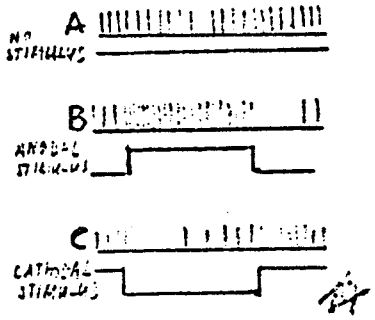
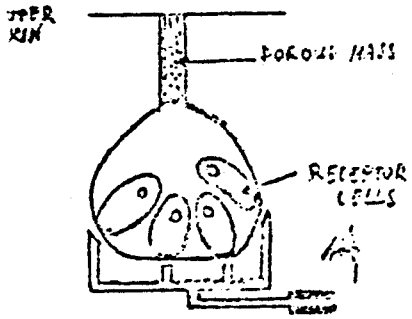


Fig. 60 Stimulus and recording from an ampullary tonic electroreceptor.



61 Tuberous phasic electroreceptor. (Schematic)

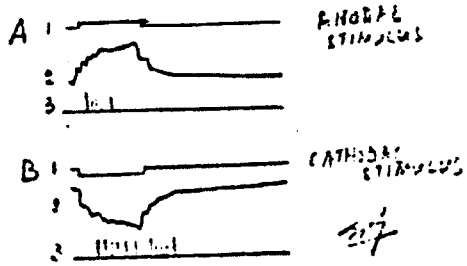


Fig. 62 Stimulus and recording from a tuberous phasic electroreceptor.

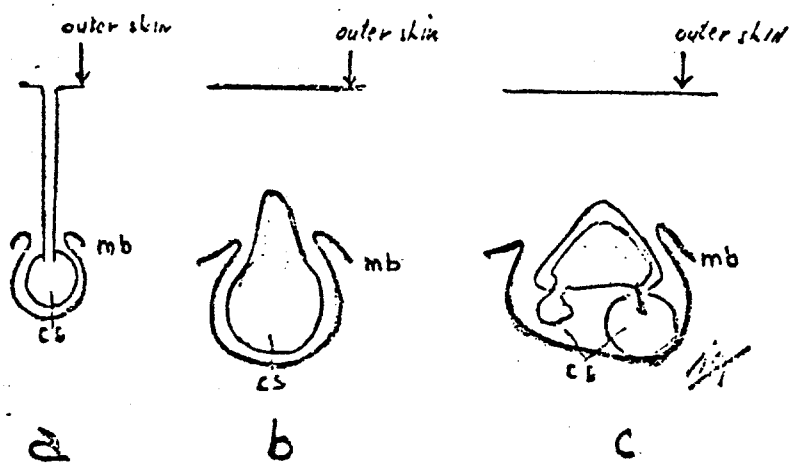
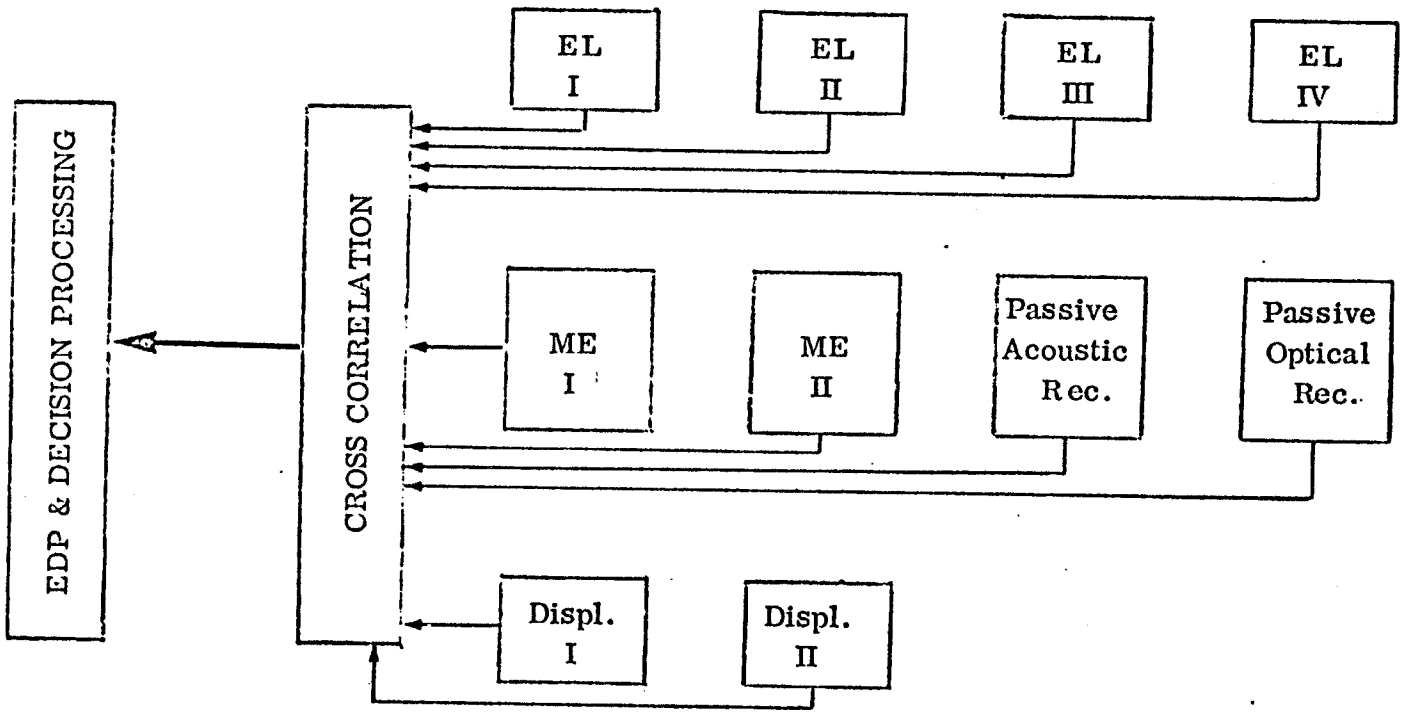


Fig. 63. Electroreceptors of Gymnarchus niloticus.

a: ampullary electrorcceptor b: tuberous electroreceptor type b
c: tuberous electroreceptor type c. (Schematic)

FIGURE 64

PASSIVE HYBRID UNDERWATER PATTERN RECOGNITION SYSTEM



EL I to IV = ELECTRIC SENSORS

ME I, ME II = MECHANICAL SENSORS

DISPL I, DISPL II = DISPLACEMENT SENSORS

ick and thin fibers. The specialized lateral line organs (electric sensory organs) be subdivided into two main groups: ampullary organs and tuberous organs. :e are many subdivisions of these organs and we have previously reported about ı.

different lateral line organ types, canal organs, free neuromasts, ampullary ns and tuberous organs, have a characteristic distribution pattern being similar ymnotids, except for Electrophorus electricus. The density of each type of organ ɔ same species depends upon the size of the specimen, e.g., canal organs in the region are separated by 1 mm in a Sternarchus albifrons 13 cm long, and by 2 mm e 22 cm long.

ıpproximate distribution of single organs was established by observing the "pore rn" on the surface of the skin with a dissecting microscope. It follows from the ous descriptions of these organs that these "pores" should not be considered as 1 hole in the integument in every case, but rather a local differentiation of the rmis overlying the sensory organs.

V. RESULTS AND CONCLUSIONS

ɔe was to develop the necessary instrumentation for microelectrode recordings
ctroreceptors of electric fish so as to be able to investigate their pattern
ion ability. In the previous chapter we demonstrated that the system is work-
and we succeeded in recording the autonomous autorhythmic electrical activity
nic ampullary electroreceptors of Sternarchus albifrons compared with the
organ's normal activity (Figures 65 and 66).

ɔrding was made during resting of the fish with no stimulus. The impulses
egular around a repetition rate of between 100 and 300. The amplitude was
.5 mv per spike. The spike duration was very short — around 200 micro-
(Figures 65, 66).

r investigation, it can be concluded that electric fishes could use their elec-
ins (transmitting and receiving) for navigation and communication — in other
ɔattern recognition.

ɔrdings and histological evidence show that Sternarchus albifrons has three
electroreceptors: ampullary tonic nonsynchronous units, ampullary tonic
ious units, and tuberous phasic nonsynchronous units. The physical analogs
ind phasic electroreceptors are shown in Figures 57 and 58. Both are repre-
y a generator connected to resistances and capacitances in series and in

The difference between tonic and phasic electroreceptors is that the first
e one resistance in series with the generator whereas the phasic electro-
s have a capacitance. The tonic electroreceptors seem to be predominant,
ke five-to-one, compared to the phasic electroreceptors. The electrore-
seem to act, to a certain extent, independently of the main electric transmitting
least two out of three different types of electroreceptors are asynchronous

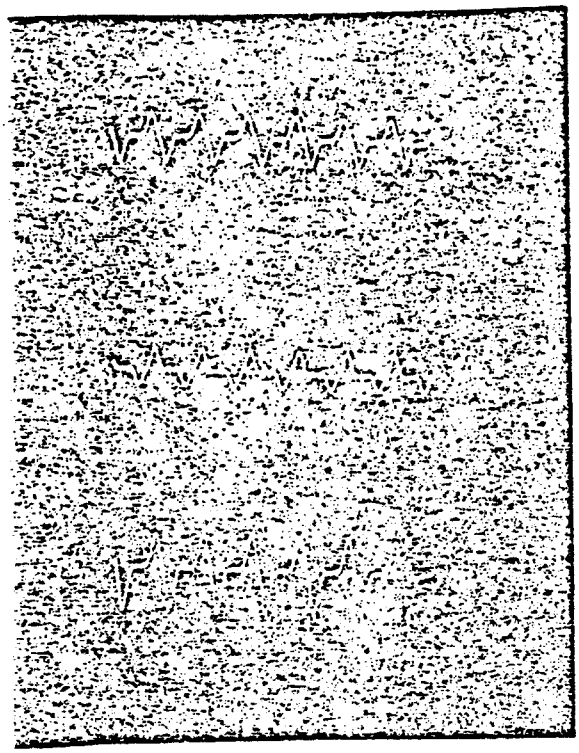
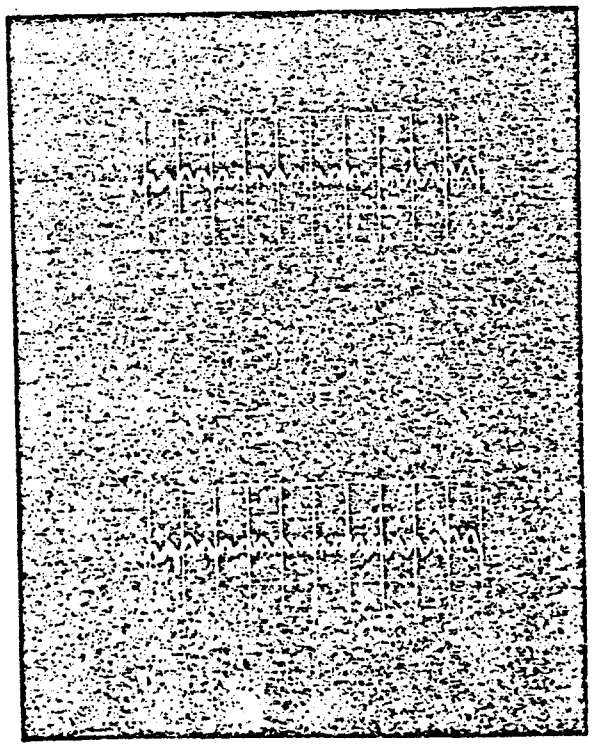


Figure 65. Recording from an anaesthetized, curarized Sternarchus albifrons specimen. Horizontal: 1 graduation = 1 msec. Vertical: 1 graduation = 10 mV.

igure 66. Microelectrode recording of
ıe autorhythmic electrical activity of
ıe ampullary, tonic electroreceptors of
ternarchus albifrons. The spikes seen
n the top of the rhythmic almost
inusoidal waveform are the electric
ignals from the electroreceptors.
orizontal: 1 graduation = 2 msec.
ertical: 1 graduation = 500 mV.
mplification x100, effective 1 gradua-
ion = 5 mV. Spike app. 2 to 2.5 mV.



ne type of electroreceptor will synchronize with the main electric organ. ound that the complete denervation of the transmitting electric organ does e activity of the asynchronous electroreceptors (both phasic and tonic). The l capable of responding to conductive and nonconductive objects placed near ody. It may affect the total capability in determining certain movements or a certain extent, its sensitivity in pattern recognition. Some of the syn- onic units are connected to one and the same nerve trunk part of the acoustico- stem but connected to specialized big nuclei in the brain.

triking fact about fresh water weak electric fish, besides their spontaneous gan, is that all of them are provided with a highly developed lateralis line elated to this acoustico-lateralis system is an enlargement of the cere- ecially in Gymnarchus niloticus and in mormyrids. The unusual importance alis system in these fish, compared with other teleosts, is not due to an in- nber of "ordinary" lateral line sensory organs, but rather to the existence umber of specialized sensory organs within this same system.

orting our hypothesis about a hybrid complex underwater pattern recognition l by electric fishes in recognition of prey, predators, and navigation in is recommended that the other lateralis line systems from different fresh electric fishes should be studied with the aim to find out the role of the sory organs in pattern recognition.

of the electric fish pattern recognition system would make it possible to models of the physical analogs of the sensors could be integrated in object location, detection and identification. The range and sensitivity of the l be assessed and improvements could be made.

ely used anaesthetic,— "MS 222" or "Finquel" (tricaine methanesulfonate) — cts the repetition rate of the electric impulses of the electric organ. It o do a series of experiments on different anaesthetics to establish whether

:e is one which would not affect the frequency of the impulses. It has been found
: thiopental sodium (sodium penthotal) will not affect the frequency of the impulses
is a safe anaesthetic for fish, acting fast and without any ill effects.

er subdivisions exist between the one and the same type of electroreceptor, but this
not been as yet investigated in a detailed way. The connections between the electro-
eptors, the different nerves, nerve-trunks and the brain have to be investigated. In
way, their interrelationship could be established. Microelectrode recordings from
electroreceivers proper and from their nerve fibers have been planned. Electro-
section and clearing of the lateral line near the electroreceptors to be investigated
enable us to record from the efferent nerve fibers.

m the experiments with Gymnarchus niloticus, we concluded that one fish would
ognize and communicate with another one of the same species. Behavioral experi-
its in this direction will be continued. It is recommended that further micro-
:trode recordings be made from the electroreceptors and from the nerve fibers of
marchus albifrons and of the newly received Gymnarchus niloticus.

s possible to simulate an equivalent sensory system responding to different stimuli
erwater. A system with a double feedback mechanism can be envisaged: (1) one
resented by a constant frequency electric field transmitting system operating on
phase-synchronous electroreceptors responding to discontinuities in the electric
d or to changes in the phase relationship transmitter-receptor; and (2) another
represented by a variable frequency transmitting system responding to disturbances
he field between transmitter and receptor with a change of the frequency of the
smitting electric organ. To this we could add an independent dual autorhythmic
eptor system: (a) responding with the increase or decrease of the autorhythmic
juency depending on movement direction of the disturbance in the electric field;
(b) responding with a change in the latency depending on the magnitude of the dis-
ance, also distinguishing between conductive and nonconductive objects.

mnarchus niloticus, one of the most sensitive electric fishes, served as a model for simulation of a simple pattern recognition system (Figures 67 and 68).

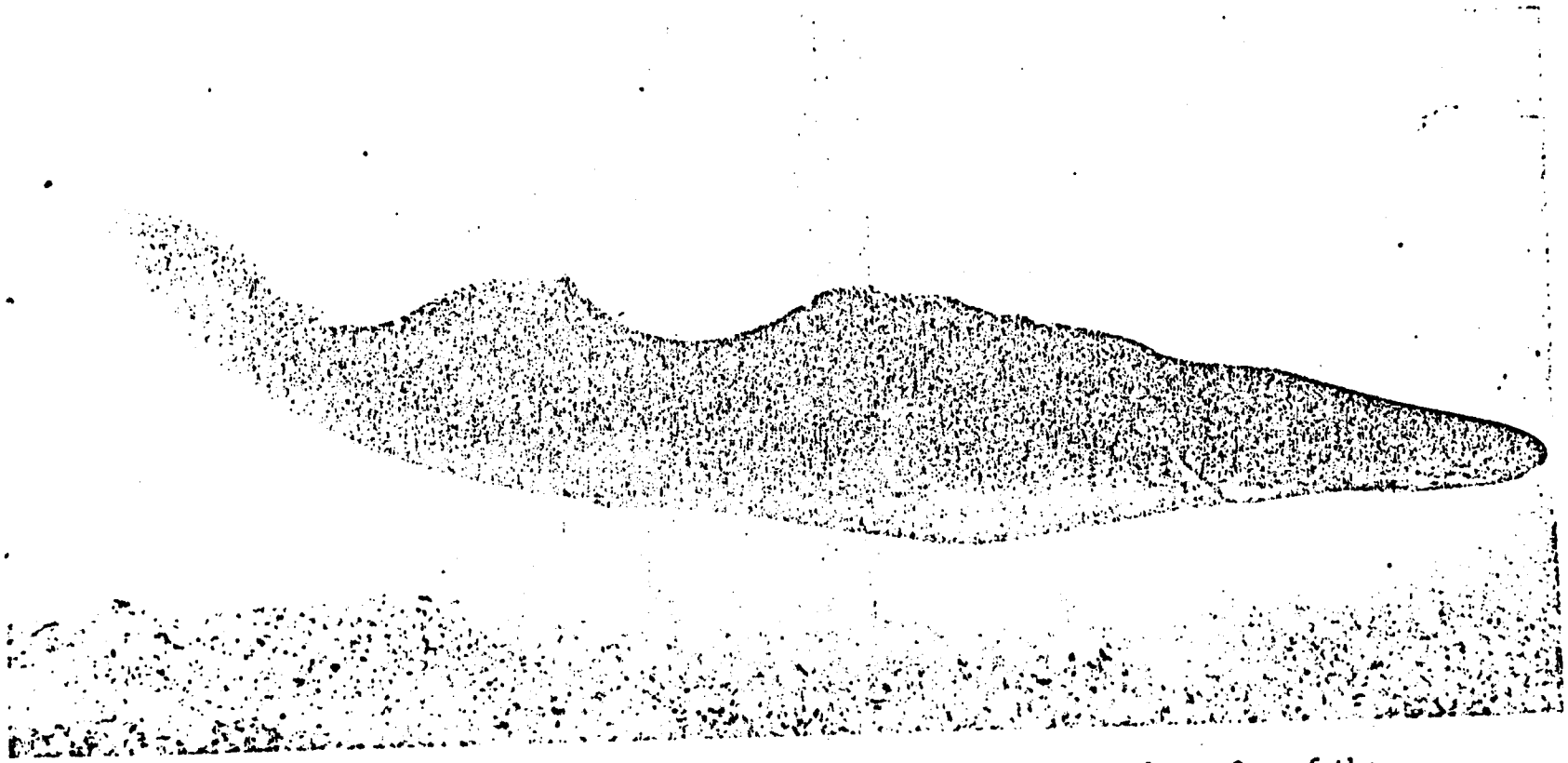
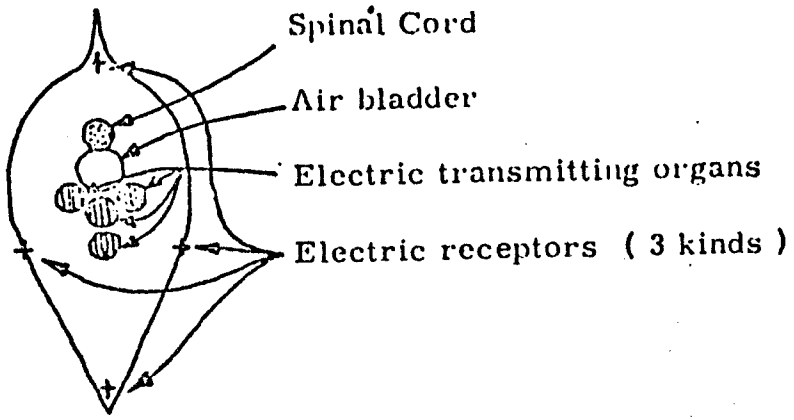


Figure 67. Gymnarchus niloticus. Country of origin: Sudan. One of the Most Sensitive Electric Fish. (Half-scale)



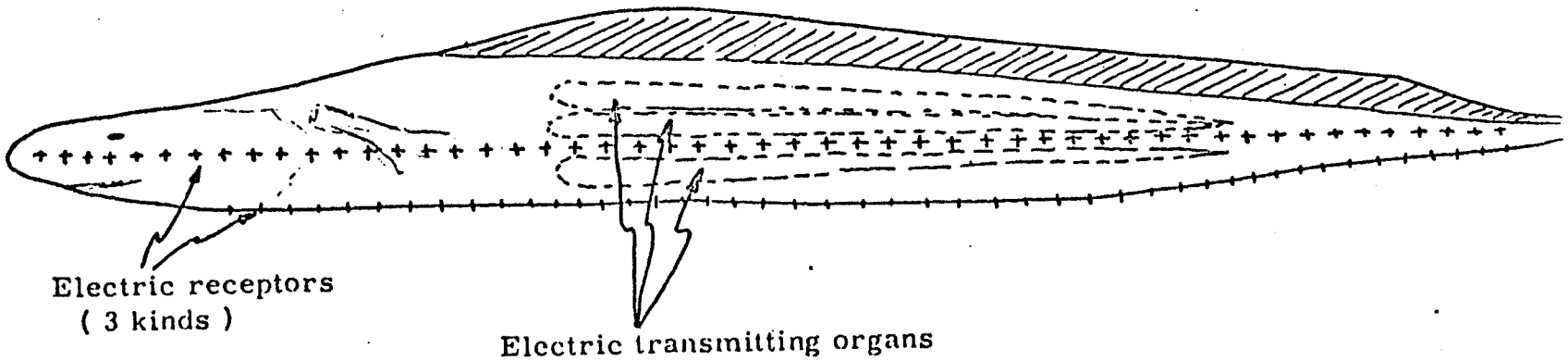


Figure 68. Gymnarchus niloticus (Electric Fish). Placement of Electric Transmitting and Receiving Organs.

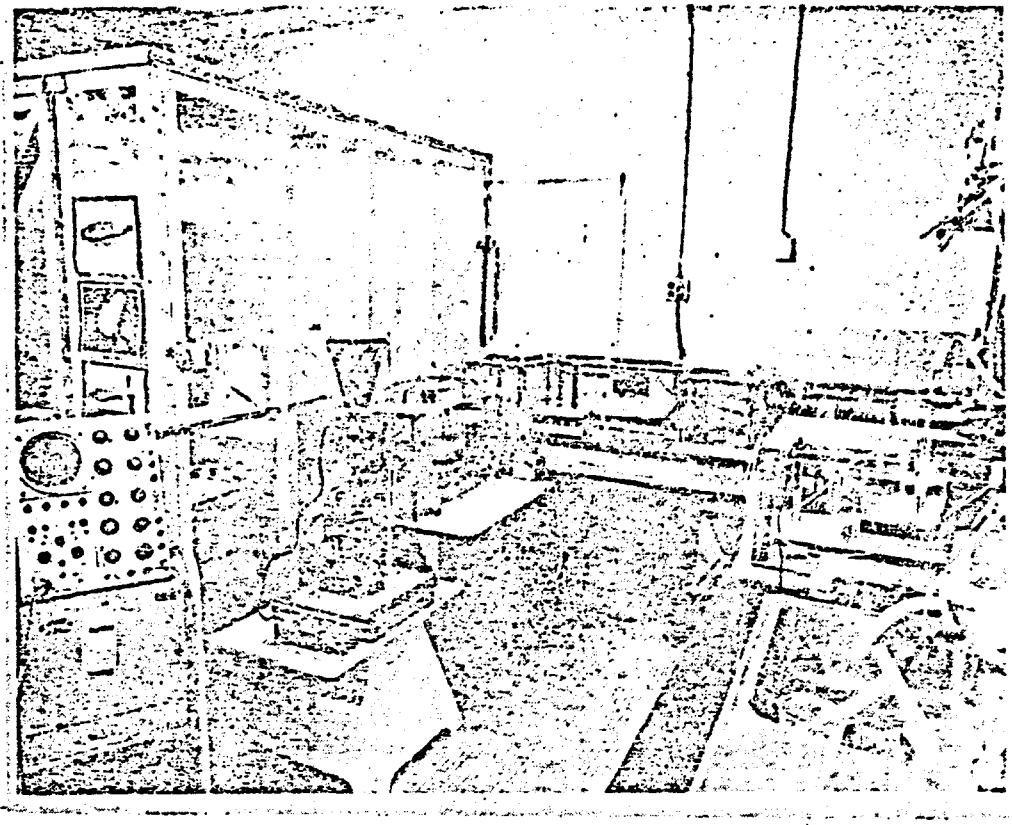


Fig. 69 Laboratory for investigation of electric fishes A.

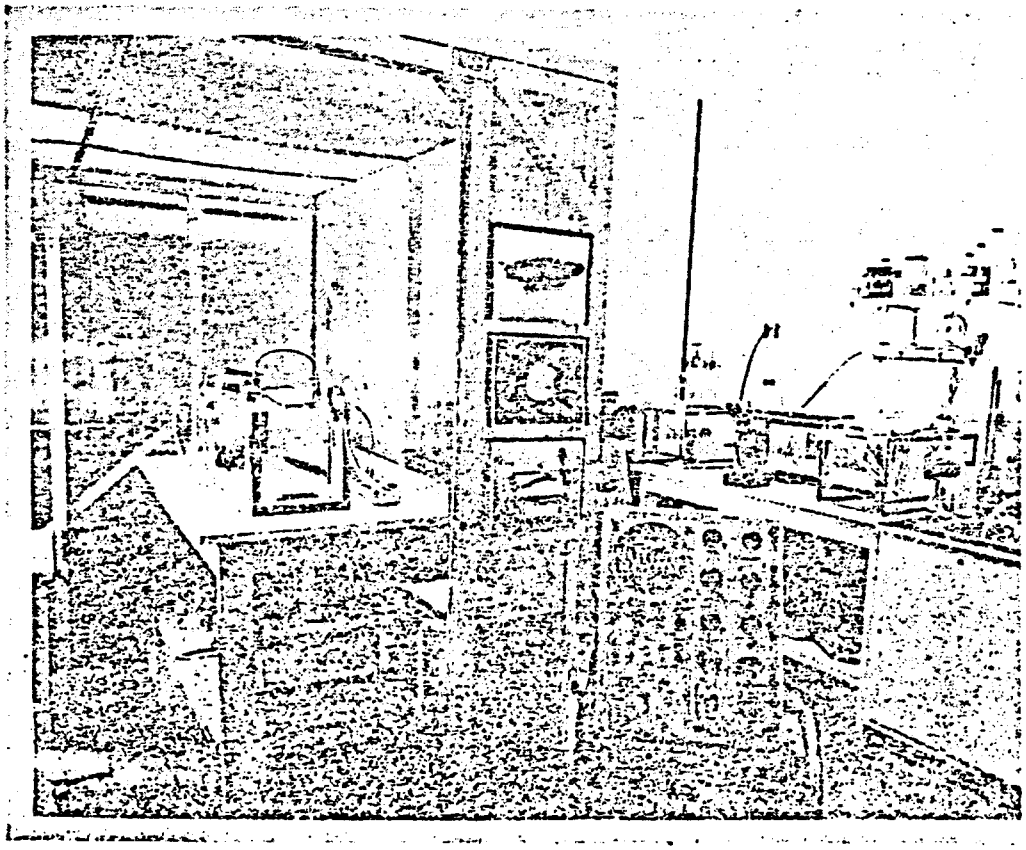


Figure 70 Laboratory for investigation of electric fishes B.

VI. REFERENCES

Watanabe, A., and K. Takeda, J. Exp. Biol. 40:57 (1963).

Bennett, M. V., Modes of Operation of Electric Organs. Annals of the New York Academy of Science 94:2:458 (1961).

Eccles, J. C., P. Fatt, and K. Koketsu, J. Physiol. 126:524 (1954).

Rose, J. E., and V. B. Mountcastle, Bull. John Hopkins Hosp. 94:238 (1954).

Grundfest, H., Progress in Biophysics 7:1-85, Pergamon Press, London, G.B. (1957).

Furukawa, J., and Furshpan, E. J., Two inhibitory mechanisms in the Mauthner neurons of goldfish, J. Neurophysiol. 26:140-176 (1963).

Diamond, J., and Yasargil, G. M., Synaptic function in the fish spinal cord, Dendritic integration. Prog. Brain Res. 31:201-209 (1969).

Auerbach, A. A. and Bennett, M.F.L., Chemically mediated transmission at a giant fiber synapse in the central nervous system of a vertebrate, J. Gen. Physiol. 53:183-210 (1969).

Auerbach, A. A. and Bennett, M.V.L., A rectifying synapse in the central nervous system of a vertebrate. J. Gen. Physiol. 53:211-237 (1969).

Agalides, E. and Sparks, S., The compound eye of arthropods in "Research and Development" GDPD General Dynamics, pp. 317-322 (1968).

Hartline, H. K., Wagner, H. G., and Ratcliff, F., Inhibition in the eye of Limulus. J. Gen. Physiol. 39:651-673 (1956).

Lissman, H. W., and Mullinger, A. M., Organization of ampullary electric receptors in Gymnotidae (Pisces), Proc. Roy. Soc. 3169:345-378 (1968).

Szabo, T., Sense organs of the lateral line system in some electric if the Gymnotidae, Mormyridae, and Gymnarchidae. J. Morphol. 117:229-250 (1965).

6. Szamier, R. B. and Wachtel, A. W., Special cutaneous receptor organs of fish. VI. The tuberous and ampullary organs of Hypopomus. J. Ultrastruct. Res. 30:450-471 (1970).

7. De Oliveria Castro, G., Morphological data on the brain of Electrophorus electricus (L) in Bioelectrogenesis (C. Chagas and A. P. de Carvalho, eds.), pp. 171-182, Elsevier, Amsterdam (1961).

8. Bennett, M. V. L., Mechanism of electroreception. In "Lateral Line Detectors" (P. Cahn, ed.), pp. 313-393, Indiana Univ. Press, Bloomington, Indiana (1967).

9. Suga, N., Electrosensitivity of canal and free neuromast organs in a gymnotid electric fish. J. Comp. Neurol. 131:453-458 (1967).

10. Bennett, M.V.L., Similarities between chemically and electrically mediated transmission. In "Physiological and Biophysical Aspects of Nervous Integration." (F.D. Carlson, ed.) pp. 78-128, Prentice Hall, Englewood Cliffs, New Jersey (1968).

21. Mullinger, A. M., The organization of ampullary sense organs in the electric fish Gymnarchus niloticus, Tissue Cell 1:31-52 (1969).

22. Lissman, H. W., and K. E. Machin, J. Exp. Biol. 35:451-486 (1958).

23. Bullock, T. H. Personal communication (1969).

24. Szabo, T. and Fessard, A. E., J. Physiol., Paris. 57:343-360(1965).

25. Bullock, T. H. and Chichibu, S., Proc. National Acad. Sci. USA. 54:422-429 (1965).

26. Agalides, E. Evolution of electric organs in fish, information processing by electric fish and communication between electric fish. Lecture at the Arthur D. Little Co., Cambridge, Mass. (21 Feb. 1961).

27. Agalides, E., D. Heppner and C. Claysmith. Communication techniques to resist jamming. GDCA-ERR-1717, Convair Aerospace Div. (October 1972).

28. Hubel, D. H., Sciences, Vol. 125, pp. 549-550 (1957).

29. Wolhbarsht, M. L., MacNichol, E. F., Jr., and Wagner, H. G., Science, Vol. 132, pp. 1309-1310 (1960).

Grundfest, H., Scngstaken, R. W., Oettinger, W. H., and Gurry, R. W., Rev. of Sci. Instr., Vol. 21, pp. 360-361 (1950).

Donaldson, P.E.K., Electronic Apparatus for Biological Research, Butterworths Sci. Publ., London, pp. 568-581 (1958).

Wood, E. M., Urethane as a carcinogen. Progressive Fish-Culturist, Vol. 18, No. 3 (1956).

Ball, J. N., and Cowen, P. N., Urethane as a carcinogen and as an anaesthetic for fishes. Nature, Vol. 184, p. 370 (1959).

Bove, F. J. MS-222 Sandoz, the anaesthetic of choice for fish and other cold-blooded organisms. Sandoz News No. 3 (1965).

Harking, L. L., Investigations in Fish Control: 12. Toxicity of MS-222 to selected fishes. U.S. Bureau of Sport Fisheries and Wildlife Resource Publication No. 18 (1966).

Walker, C. R., and Schoettger, R. A., Investigations in Fish Control: Residues of MS-222 in four species of salmonids following anesthesia. U.S. Bureau of Sport Fisheries and Wildlife Resource Publication No. 21 (1966).

Schoettger, R. A., and Julin, A. M., 13. Efficacy of MS-222 as an Anesthetic on Four Salmonids. U.S. Bureau of Sport Fisheries and Wildlife Resource Publication No. 19 (1967).

Clontz, G. W., "Anesthesia of Fishes", in Experimental Animal Anesthesiology by D. C. Sawyer, AD 648 329, U.S. Dept. of Commerce 1967 Symposium held Dec. 1964 at the Brooks AFB, Texas.

The MERCK INDEX.

Szabo, Th., in Lateral Line Detectors (P. Cahn, ed.), pp. 295-311, Indiana University Press (1971).

Hagiwara, S., Szabo, Th., and Enger, P.S., J. Neurophysiol. 28:775-783 (1965).

Enger, P.S., and Szabo, Th., J. of Neurophysiol. 28:775-783 (1965)

Kobua Suga., J. of Comp. Neurol. 131:4:437-451 (1967).

Bullock, T. H., Brain Behav. Evol. 2:85-118 (1969)

Hartop: U. S. Patent 3109001 to Abbot Labs (1963).

Kalmijn, E., Sensitivity and Behavioral Reaction of Sharks to Electric Stimuli, Final Report. ONR Contract Nonr 4773(00) No. 104-863 (1967).

Hagiwara, S., Szabo, T., and Enger, P.S., Electroreceptor mechanism in a high frequency weakly electric fish, Sternarchus albifrons. J. Neurophysiol. 28:5:784-799 (1965).

Szabo, T., Nature, 194:4848:600-601 (1962).